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Abstract

Proceedings from the 2016 American Elm Restoration Workshop in Lewis Center, OH. The published proceedings include 16 papers pertaining to elm pathogens, American elm ecology, and American elm reintroduction.

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Cover Photo

Baldwin Hill elm, late summer, 2013. Baldwin Hill crests between North and South Egremont in the southern Berkshires of western Massachusetts. The elm is growing on conservation farmland and was the first heritage American elm protected by the Elm Watch Adopt an Elm program. ©Tom Zetterstrom 2013, used with permission.

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General Technical Report NRS-P-174

Foreword

On three beautiful fall days in central Ohio, an interdisciplinary group of scientists from across the United States and Canada came together at the American Elm Restoration Workshop to discuss past, present, and future research on American elm restoration (see page 144 for list of workshop participants). From October 25 to 27, 2016, the researchers met at Highbanks Metro Park in Lewis Center, OH, to capture historical insights into Dutch elm disease (DED) and the American elm, review the current status and evaluate the goals of future research, and foster collaboration among elm scientists. Of particular interest was garnering unpublished knowledge from scientists who were part of the "second front" of American elm research, circa 1960–1990, which benefited from substantial Federal funding. Capturing insights and knowledge from soon-to-be- and already-retired scientists was thus a primary goal of this workshop. The USDA Forest Service Northern Research Station and State & Private Forestry jointly hosted this workshop, with generous support from the Manton Foundation.

The workshop covered a variety of topics relating to American elm restoration, including the DED pathogen, DED tolerance, genetics and ecology of the elm hosts, other threats to American elm, other tools to combat DED, and practical aspects of restoration. Together, the workshop presentations, partially represented by the papers in these proceedings, described challenges to American elm restoration, recent advances in the field, and research needs.

As an introduction to the workshop, James M. Slavicek began with a description of an American elm restoration project involving the USDA Forest Service Northern Research Station and The Nature Conservancy. Some aspects of this project include screening survivor and progeny elms, represented in the paper by Charles E. Flower and colleagues (p. 24). Michael Marcotrigiano laid the groundwork for discussions with an overview of the research that has been done on DED and American elm, highlighting many of the answered and unanswered questions (p. 2). Louis Bernier helped us to focus briefly on the DED pathogen and warned of the potential of *Ophiostoma* spp. to overcome DED tolerance in DED-tolerant American elms, as well as the potential for the pathogen to jump to new insect vectors (p. 6).

We proceeded to discuss the response of the elm host to DED through a series of talks on tolerance. Slavicek shared data that demonstrated heritability of DED tolerance. Crosses among DED-tolerant trees produced progeny with a range of tolerance phenotypes, including a large number of trees with excellent tolerance. Bernier provided information on several disease-response genes that are present in DED sensitive American elm but perhaps activated too late during pathogenesis to prevent disease. There are many traits that could be exploited in developing resistance, including anatomical features (size and spatial organization of vessels), timing of leaf flushing, preformed resistance molecules (phytoanticipins), induced resistance molecules (phytoalexins) and proteins, and attractiveness to elm bark beetles. Sherif M. Sherif shared data (published elsewhere) on activation of genes, demonstrating that tolerant American elms express certain genes more quickly and with greater magnitude than susceptible elms. Jasmonic acid is involved, and the response differs among tolerant elms, indicating multiple defense mechanisms. Alan T. Whittemore provided insight into the variable ploidy levels observed in American elm, alluding that the recently discovered diploid American elm that is more common in the southeastern United States may actually be a cryptic species and could provide a source of DED tolerance. Garrett L. Beier shared some preliminary results from his PhD research on compartmentalization response of American elms to inoculation with *Ophiostoma novo-ulmi*, demonstrating that some putatively tolerant clones show differential formation of zone 3 and zone 4 defense barriers.

We followed these presentations with a panel discussion on methods used for challenge inoculations. Consistency and effectiveness in screening is necessary for accuracy and comparability of results. A summary of key observations from this panel are captured in these proceedings (Haugen et al, p. 37). Additional insights on how elm response to inoculation is affected by many factors, including time of year, age of tree, soil moisture, source and amount of inoculum, and method of delivery are provided in the papers by Beier et al. and Flower et al. in these proceedings (pgs. 30 and 24, respectively).

We completed our topic on DED tolerance with a presentation by Tom Zetterstrom and colleagues about shortcomings in commercially-available elms, either in DED-tolerance or form (p. 119). Chad P. Giblin et al.'s paper provides further evidence of the failure of widely-planted cultivars in urban settings due to weak branch or leader attachments, unrelated to DED tolerance (p. 122). We also held an open discussion on the availability and characteristics of specific clones, as captured in the paper by Linda M. Haugen and Susan E. Bentz (p. 109).

On the second day of the workshop, we resumed our consideration of the elm host with a series of talks more related to the importance of elms in the broader landscape. Jennifer L. Koch presented a summary of FIA data, which shows that in areas with longest presence of DED, some larger elms are persisting on the landscape. This persistence of large elms is not occurring in states where elm yellows is common, indicating that elm yellows may be contributing to the mortality of larger elms. Johanne Brunet found that the arrival of DED to North American has not reduced genetic diversity among slippery elm (*Ulmus rubra*), likely due to the high gene flow facilitated by wind-dispersed pollen and low levels of inbreeding, and suggested that DED is similarly unlikely to greatly diminish the genetic diversity of American elm (p. 99). Whittemore shared an analysis of the phylogeny of all known elm species, showing that our native North American elms (with the exception of *Ulmus rubra*) are in the subgenus *Oreoptelea*, whereas most of the "Old World" elms are in the subgenus *Ulmus*. It appears that several other species of elm could provide useful germplasm for breeding for resistance to DED and other valuable traits, particularly Chinese *U. elongata* and Himalayan *U. villosa.*

As we shifted our consideration to other elm problems, Gary W. Moorman provided an overview of the elm yellows epidemic which occurred at Pennsylvania State University, and the lessons learned. There are three known strains of elm yellows: the European, common, and Illinois strains; the Pennsylvania outbreak was caused by the common strain. They observed that DED outbreaks often follow elm yellows, as the declining elms lead to increase in the elm bark beetles, which leads to an increase in DED. Cristina Rosa worked on identifying the insect vectors, and characterizing the elm yellows population, and her novel insights are captured in this proceedings (p. 49). In addition, Flower et al. recounted the complications of "false positives" that may be obtained using current molecular tools, because of presence of similar size and sequence DNA from other common bacteria (p. 68).

Despite the many challenges to successfully growing American elm, there are useful tools to manage DED in high value trees. William (Bill) L. MacDonald described a case study that demonstrates the value of chemical injection to preserve high value elms (p. 43). R. Jay Stipes prepared a summary of management options for this proceedings (p. 21).

On the afternoon of the second day, we were able to tour the USDA FS Northern Research Station field site in Delaware, OH, to observe their current American elm research and have additional discussions. The tour included field experiments to test survivor elms and

crosses for DED tolerance, as well as demonstrations of techniques including propagation by cuttings, controlled pollinations, and fungal inoculation. In addition to viewing the field, laboratory, and greenhouse elm research procedures, Bruce R. Fraedrich, Chad P. Giblin, and Tom Zetterstrom shared insights on recognizing and correcting defects in the form of DED tolerant clones. Some of these insights are captured in the paper by Chad P. Giblin et al. in this proceedings (p. 122).

On the final day of our workshop, we discussed topics related to elm restoration on the landscape. Christian O. Marks presented a comprehensive evaluation of American elm ecology, in the context of why it is important to restore and reintroduce this species (p. 74). Because American elm was a dominant species in many floodplain forests of the Midwest and Northeast, it can be considered a foundational species. Due to the functional redundancy provided by other species common to the same ecosystems, such as *Acer rubrum* and *Fraxinus* spp., the loss of American elm may not have had as large of an effect on ecosystem processes as has the loss of other foundation species. However, with the landscape-wide mortality of ash species due to emerald ash borer, that redundancy is diminished and the consequences of the loss of those species exacerbated. This was followed by a review by Kathleen S. Knight and colleagues of the current efforts to evaluate methods for reintroducing American elm to natural areas both in the context of species and ecosystem restoration (p. 133). Numerous studies have recently been established to evaluate various aspects of reintroduction, from stock type of American elm planted, to shade-, cold-, and flood-tolerance of the planted elm to the utility of American elm in ecosystem restoration treatments (also described in Flower et al., p. 141).

The papers contained in these proceedings illustrate the breadth and depth of the expertise that was represented at the workshop. While some of the valuable discourse from discussions, panels, and the field tour is not captured in this proceedings, the ongoing conversations and collaborations initiated at this workshop may benefit elm research for years to come. These papers show how far American elm research has come, as researchers have made substantial progress in combatting Dutch elm disease, and are now embarking on better understanding elm yellows. While there is a long way to go to fully address the threat of these diseases and restore American elm in forested and urban areas, the enthusiasm, determination, and multidisciplinary expertise of current researchers provides hope for all who love the American elm.

–The editors,

Cornelia (Leila) C. Pinchot, Kathleen K. Knight, Linda M. Haugen, Charles (Charlie) E. Flower, and James (Jim) M. Slavicek

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ELM PATHOGENS

ELMS AND DUTCH ELM DISEASE: A QUICK OVERVIEW

Michael Marcotrigiano[1]

In the 1930s Dutch elm disease (DED) was accidentally introduced from Europe into the United States. It had a devastating impact on American elm (*Ulmus americana*) and its relatives in urban and riparian environments. In the United States, the three-part pathosystem for DED is unique in that the affected elm species are North American, the pathogen originated in Asia, and the most common vector is a European beetle. Of the nearly 40 species of elms that span the globe from Asia to Europe to North America, European and North American species are the most DED susceptible. The disease outbreak was extremely costly and the scientific and regulatory reaction to the issue was interrupted by World War II, which allowed the disease to spread more rapidly (Campanella 2003).

DED initiated a burst of activity in the elm research community. Selection and breeding programs aimed at DED tolerance were initiated. The earliest began in the 1930s with collaboration between the Boyce Thompson Institute (Ithaca, NY) and Cornell University with the intentional infection of more than 20,000 elm seedlings with a goal of isolating resistant survivors (Sinclair et al. 1974). The results were disappointing as nearly 40 years later only 16 trees survived. All of the survivors had undesirable characteristics, and none of their tested offspring were DED tolerant. Breeding programs that began by crossing DED-tolerant American elms were initiated at the University of Wisconsin (UW) (Guries and Smalley 1990) and the U.S. Department of Agriculture National Arboretum (NA) (Townsend 2000). Elms that tested DED tolerant began to be released to the trade over the next several decades. These include the Liberty multiclone (UW), 'Valley Forge' and 'Jefferson' (NA), and 'New Harmony' (NA). After a long gap, other institutions have recently released more clones. Some of these clones are from sole survivors programs that resulted from exploration to find old American elms. Because such survivors often succumb to the disease later, and because the great majority of survivors that have been inoculated with the fungus show little or no disease tolerance, it has been argued that most sole survivors merely escaped infection (Becker 1996). Relative testing of the newer clones for DED tolerance against older DED-tolerant clones has yet to be reported. In addition to the NA and the UW, the Morton Arboretum (MA) in Illinois utilized Asian elm species in hybridization to generate useful urban elms that are DED resistant. Some of the more widely used examples are *Ulmus* 'New Horizon' (UW), 'Morton' (Accolade®), 'Morton Glossy' (Triumph®) (MA), and 'Frontier' (NA), although many more are commercially available. The National Elm Trial (Colorado State University, n.d.), now complete, will soon publish comprehensive findings on the performance of many hybrids in different regions as well as the performance of some American elm selections. Recently, it has been discovered that *U. americana*, once thought to be the only tetraploid species in the genus *Ulmus*, also exists in the diploid state in some U.S. populations (Whittemore and Olsen 2011). The significance of this discovery with regard to DED tolerance, breeding, and taxonomy is yet to be determined.

Much research has been done to try to understand what makes an elm tolerant of DED. The cycle of pathogenicity is well understood. Fungal spores (largely from the fungus *Ophiostoma novo-ulmi*) carried by beetles enter a wound generated by beetle activity. After spores germinate, the fungus begins to dissolve the cell walls and feeds on plant carbohydrates. Embolisms in the xylem occur and the tree reacts by making suberin and lignin and attempts to localize the pathogen by blocking vessels and tracheids with cell wall extensions called tyloses. If this does

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not occur rapidly (as in DED-susceptible clones) the xylem become ineffective and the tree cannot properly hydrate. Defense anatomy studies indicate a correlation between vessel size and susceptibility (Elgersma 1970, McNabb et al. 1970) but recently, other hydraulic parameters have been implicated (Martin et al. 2013). In addition, the time a tree breaks bud in the spring can affect its susceptibility (Ghelardini and Santini 2009). From a genetic standpoint, it is accepted that the inheritance of DED tolerance is complex and multigenic (Aoun et al. 2010, Townsend 2000). Elm defense chemistry is multifaceted and involves the biosynthesis of many compounds that act directly or in complex pathways (reviewed by Büchel et al. 2016). In summary, the elm defense system is a generalized one that aims at walling off the infected xylem to prevent the fungus from infecting new growth, and is controlled by many genes. Temporal and phenological aspects of *Ulmus* growth play a major role in susceptibility.

The first DED fungus to be found in the United States was the less aggressive *Ophiostoma ulmi* (Brasier 1991). By 1940, this species was largely replaced by the more aggressive *O. novo-ulmi* (Brasier 1991). A third species, *O. himal-ulmi*, has been identified as a naturally occurring endophyte on elms native to the Himalayas and the elms are largely asymptomatic. However, when European elms are purposely inoculated with it, it is pathogenic (Brasier and Mehrotra 1995). From a genetic standpoint, fungi are easier to analyze than trees or insects and their smaller genome, short life cycle, and ability to be grown in culture makes them easier targets for genetic dissection. DNA sequencing has been completed on the genome of *Ophiostoma novo-ulmi* (Forgetta et al. 2013) and *O. ulmi* (Khoshraftar et al. 2013). For more information on *Ophiostoma* see a recent review (Bernier et al. 2015).

With regard to the fungal vector, it appears that any insect that can place DED spores into a stem wound can accomplish inoculation, although in the eastern United States the bark beetles belonging to the genus *Scolytus* are the main vectors (Santini and Faccoli 2014). We now know that the DED fungus can attract more beetles by emitting certain volatiles (McLeod et al. 2005) and that the bark beetles can be infected with a certain mite that has sporotheceae, which can increase beetle spore load (Moser et al. 2010).

Studies and breeding of American elm have focused much more on DED and less on elm yellows (i.e., elm phloem necrosis), which is caused by a phytoplasma. This is a concern as elm yellows is lethal. There are many subgroups of this pathogen as indicated by genetic sequencing (Jović et al. 2011). There is no practical treatment or cure. Control would involve an aggressive pesticide program to kill the vectors, of which there are many leafhopper species. Another strategy to combat tree diseases is to generate genetically engineered trees. Genetic engineering for DED resistance in American elm has been accomplished with significant reduction in pathogen symptoms (Gartland et al. 2005), but concerns over regulatory issues and a lack of funding[2] have slowed down progress.

After reviewing the elm disease literature, many questions and issues remain and it is hoped these will be answered with future research. For breeders, an image database of mature examples of all elm species would be useful to ascertain their aesthetic value. In addition, trials that rank the performance of elms intended for landscape use have been done in fields, not cities. How would the relative performance of these elms be in urban settings where trees are exposed to road salts, drought, and restricted root areas? With about 35 elm species, most never utilized in breeding or genetic analysis, is there more opportunity for interspecific hybridization to produce new landscape elms? Will diploid American elms alter our understanding of American

[2] Personal communication from William A. Powell, Professor and Director, Biotechnology in Forestry, State University of New York, Syracuse.

elm evolution, DED, or assist breeders in any way? What role does the DED fungus play in Asia where it is not a pathogen? American elms have played a key role in urban planning and in natural ecosystems. Arguments for funding research aimed at restoring this species are defensible.

A more comprehensive review (Marcotrigiano, in press), which also includes a list of elm germplasm available in the United States, has been accepted and will appear in the journal "Arboriculture and Urban Forestry."

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Special thanks to Susan E. Bentz (USDA Agricultural Research Service, National Arboretum) and Louis Bernier (University of Laval) for making me aware of and/or forwarding me relevant manuscripts. I appreciate the willingness of Alan T. Whittemore (USDA Agricultural Research Service, National Arboretum), Raymond P. Guries (Emeritus Professor, University of Wisconsin), Kris Bachtell (Morton Arboretum), and William A. Powell (State University of New York) to answer email queries.

Literature Cited

Aoun, M.; Jacobi, V.; Boyle, B.; Bernier, L. 2010. **Identification and monitoring of *Ulmus americana* transcripts during in vitro interactions with the Dutch elm disease pathogen *Ophiostoma novo-ulmi*.** Physiological and Molecular Plant Pathology. 74: 254-266.

Becker, H. 1996. **New American elms restore stately trees.** Agricultural Research. July 1996: 4-8. http://agresearchmag.ars.usda.gov/AR/archive/1996/Jul/elms.pdf (accessed December 30, 2015).

Bernier, L.; Aoun, M.; Bouvet, G.F.; Comeau, A.; Dufour, J.; Naruzawa , E.S.; Plourde, K.V. 2015. **Genomics of the Dutch elm disease pathosystem: are we there yet?** Journal of Biogeosciences and Forestry. 8:149-157.

Brasier, C.M. 1991. ***Ophiostoma novo-ulmi* sp. *nov*., causal agent of the current Dutch elm disease pandemic.** Mycopathologia. 115: 151-161.

Brasier, C.M.; Mehrotra, M.D. 1995. ***Ophiostoma himal-ulmi* sp. *novi*., a new species of Dutch elm disease fungus endemic to the Himalayas.** Mycological Research. 99: 205-215.

Büchel, K.; Fenning, T. ; Gershenzon, J.; Hilder, M.; Meiners, T. 2016. **Elm defense against herbivores and pathogens: morphological, chemical and molecular regulation aspects.** Phytochemistry Reviews. 15(5): 961-983.

Campanella, T.J. 2003. **Republic of shade – New England and the American elm.** New Haven, CT: Yale University Press. 240 p.

Colorado State University College of Agricultural Sciences. N.d. **National elm trial.** Ft. Collins, CO: Colorado State University. http://bspm.agsci.colostate.edu/national-elm-trial/ (accessed November 2, 2016).

Elgersma, D.M. 1970. **Length and diameter of xylem vessels as a factor in resistance of elms to *Ceratocystis ulmi*.** Netherlands Journal of Plant Pathology. 76: 179-182.

Forgetta, V.; Leveque, G.; Dian, J.; Grove, D.; Lyons, R., Jr. [et al.]. 2013. **Sequencing the Dutch elm disease fungus genome using the Roche/454 GS-FLX titanium system in a comparison of multiple genomics core facilities.** Journal of Biomolecular Techniques. 24: 39-49.

Gartland, K.M.A.; McHugh, A.T.; Crow, R.M; Garg, A.; Gartland, J.S. 2005. **2004 SIVB Congress symposium proceeding: biotechnological progress in dealing with Dutch elm disease.** In Vitro Cellular and Developmental Biology–Plant. 41: 364-367.

Ghelardini, L.; Santini, A. 2009. **Avoidance by early flushing: a new perspective on Dutch elm disease research.** iForest-Biogeosciences and Forestry. 2: 143-153.

Guries, R.P.; Smalley E.B. 1990. **Selecting and testing of elms: the Wisconsin elm breeding program.** In: Proceedings of the seventh conference of the Metropolitan Tree Improvement Alliance – METRIA 7: trees for the nineties; 1990 June 11-12; Lisle, IL. Mills River, NC: Mountain Horticulture Crops Research and Extension Center: 21-29. https://www.ces.ncsu.edu/fletcher/programs/nursery/metria/metria07/index.html

Jović, J.; Cvrković, T.; Mitrović, M.; Petrović, A.; Krstić, O.; Krnjajić, S.; Toševski, I. 2011. **Multigene sequence data and genetic diversity among '*Candidatus* Phytoplasma ulmi' strains infecting *Ulmus* spp. in Serbia.** Plant Pathology. 60: 356-358.

Khoshraftar, S.; Hung, S.; Khan, S.; Gong, Y.; Tyagi, V. [et al.]. 2013. **Sequencing and annotation of the *Ophiostoma ulmi* genome.** BMC Genomics. 14: article 162. http://dx.doi.org/10.1186/1471-2164-14-162.

Marcotrigiano, M. In press. **Elms revisited.** Journal of Arboriculture and Urban Forestry.

Martin, J.A.; Solla, A.; Ruiz-Villar, M.; Gil, L. 2013. **Vessel length and conductivity of *Ulmus* branches: ontogenetic changes and relation to resistance to Dutch elm disease.** Trees. 27: 1239-1248.

McLeod, G.; Gries, R.; von Reuß, S.H.; Rahe, J.E.; McIntosh, R.; König, W.A.; Gries, G. 2005. **The pathogen causing Dutch elm disease makes host trees attract insect vectors.** Proceeding of the Royal Society, Biological Sciences. 272: 2499-2503.

McNabb, H.S., Jr.; Heybroek, H.M.; Macdonald, W.L. 1970. **Anatomical factors in resistance to Dutch elm disease.** Netherlands Journal of Plant Pathology. 76: 196-204.

Moser, J.C.; Konrad, H.; Blomquist, S.R.; Kirisits, T. 2010. **Do mites phoretic on elm bark beetles contribute to the transmission of Dutch elm disease?** Naturwissenschaften. 97: 219-227.

Santini A.; Faccoli, M. 2014. **Dutch elm disease and elm bark beetles: a century of association.** iForest-Biogeosciences and Forestry. 8: 126-134.

Sinclair, W.A; Welch, D.S.; Parker, K.G.; Tyler, R.J. 1974. **Selection of American elms for resistance to *Ceratocystis ulmi*.** Plant Disease Reporter. 58: 784-788.

Townsend, A.M. 2000. **USDA genetic research on elms.** In: Dunn, C.R., ed. The elms: breeding conservation and disease management. Boston, MA: Kluwer Academic Publishers: 271-278.

Whittemore, A.T; Olsen, R. T. 2011. ***Ulmus americana* (Ulmaceae) is a polyploidy complex.** American Journal of Botany. 98: 754-760.

GENOME-WIDE ANALYSES OF THE DUTCH ELM DISEASE FUNGI

Louis Bernier[1]

Abstract.—The Ascomycete fungi *Ophiostoma ulmi* and *O. novo-ulmi* are the pathogens respectively responsible for the two successive pandemics of Dutch elm disease (DED) since the early 1900s. The advent of the highly fit and virulent *O. novo-ulmi* was a landmark event in the evolution of DED during the last 100 years. This, however, may not be the last major shift in the behavior of the pathogen and elm breeders must keep in mind this possibility as it bears consequences for ongoing elm improvement programs. This contribution reviews some of the current knowledge on the biology of the DED fungi, including findings from genome-wide analyses carried out during the last decade. Biological traits believed to be important for parasitic fitness of *O. novo-ulmi* are presented. Events that might allow the DED fungi to evolve further are proposed and discussed in the context of elm resistance breeding.

Introduction

Few diseases of trees have had as much impact in the last 100 years as Dutch elm disease (DED). Along with chestnut blight and white pine blister rust, DED is a textbook example of the destruction caused by fungal pathogens upon gaining access to new territories and host species. In the case of DED, two successive pandemics have killed an estimated 1 billion elms (Paoletti et al. 2006) native to Eurasia and North America, as well as elms introduced to New Zealand (Ganley and Bulman 2016). The sudden and spectacular development of the disease has led to prognostics of elm extinction or, at best, survival of elms as a scrub or understory population, with a few mature elms as escapees (Brasier 1983) in areas outside central and eastern Asia where native elm species are usually highly resistant to DED.

Breeding for disease resistance is one long-term proven approach for managing losses caused by pathogens and pests of trees. The first elm resistance breeding program was launched more than 80 years ago in the Netherlands by Christine Buisman and resulted in the selection and release of a few cultivars that were considered resistant to DED (Holmes and Heybroek 1990). Soon, however, this material had to be replaced with new cultivars as it became evident that the DED fungus had become more virulent. This situation is, of course, not unique to DED since resistance breeding faces a unique challenge as the pathogen, being a living organism with a short life cycle, has the potential to evolve and change within a few years. Nevertheless, there are many cases of successful resistance breeding programs in a wide variety of agricultural crops and forest tree species. In the case of DED, most published success stories concern European elm breeding programs, for reasons that are beyond the scope of this contribution. The 2016 American elm restoration workshop and proceedings, however, provides a unique opportunity to discuss the status of elms native to North America in the face of the ongoing pandemic of DED, revisit the genetics, biology, and physiology of elms, and showcase some of the most promising avenues for maintaining these taxa as part of the landscape. These topics will not be discussed here. Rather, the scope of this contribution is to provide an overview of the biology

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of the DED pathogens and further discuss life traits that may contribute to parasitic fitness. Focus will be placed on the potential for the DED pathogens to adapt to changes occurring in their hosts following natural selection and resistance breeding. The discussion will thus include a retrospective look at changes that have occurred in the pathogen since the onset of DED, as well as a prospective assessment of what elm breeders may be faced with in the future. Some of the information included here was already presented in more detail in previous reviews (Bernier 2016, Bernier et al. 2015). On the other hand, several ideas are discussed for the first time in this contribution, ideas inspired by the latest findings in the molecular biology and genomics of the DED fungi and related species, as well as by unpublished results from ongoing investigations.

Taxonomical Status of the DED Fungi

For many years, DED was considered to be caused by one fungal pathogen referred to as *Ceratocystis ulmi*. In the late 1980s, the fungus was reassigned to the genus *Ophiostoma* and hence became known as *O. ulmi*. In 1991, however, researchers formally recognized the occurrence of two distinct DED pathogens, *O. ulmi* and *O. novo-ulmi*. *Ophiostoma ulmi* caused the first pandemic which started in the early 1900s and lasted until the end of the 1960s in most areas impacted by DED, whereas *O. novo-ulmi* is responsible for the second, ongoing pandemic of DED which is believed to have started in the late 1940s (Brasier 1991). Two geographic subspecies, designated *novo-ulmi* and *americana,* are recognized in *O. novo-ulmi* (Brasier and Kirk 2001). As indicated above, *O. ulmi* and *O. novo-ulmi* are responsible for the drastic decline in elm populations in Eurasia, North America, and other parts of the world where elms have been introduced. A third DED fungus, *O. himal-ulmi*, has also been reported in northeast India but is not associated with a disease epidemic (Brasier and Mehrotra 1995). The DED fungi are phylogenetically related to several species of saprophytic *Ophiostoma* species, which cause sapstain (or blue stain) in various deciduous and coniferous tree species (Fig. 1).

Because of changes in the taxonomy of the pathogens, discovery of cryptic taxa, as well as use of outdated nomenclature by some authors, the DED literature can be confusing for nonspecialists. For example, North American isolates of the highly aggressive strain of *Ceratocytis ulmi* mentioned in earlier reports likely represent isolates of *O. novo-ulmi* subsp. *americana*. The latter seems to be the only taxon found in North America in the last decades (e.g., Houston 1991), whereas both subspecies co-occur in several areas in Europe (e.g., Brasier and Kirk 2010, Tziros et al. 2017).

Salient Biological Traits of the DED Fungi and Other Ophiostomatales

All DED fungi are pathogens but exhibit varying degrees of virulence (used here in a quantitative sense). *O. ulmi* is less virulent and was previously referred to as the "nonaggressive strain." Nevertheless, *O. ulmi* can kill more susceptible elm species such as *U. americana*. *Ophiostoma novo-ulmi* is highly virulent and can kill elm species that were tolerant to *O. ulmi*. The third DED fungus, *O. himal-ulmi*, was recovered from symptomless *Ulmus wallichiana*. Technically, it could be considered as an endophyte on this host but has been shown to induce typical DED symptoms when inoculated to European elms (Brasier and Mehrotra 1995).

The DED fungi are vascular pathogens that have evolved several traits (discussed below) that allow them to colonize the water-conducting vessels in the xylem. There is evidence that these pathogens also have an effect (direct and/or indirect) on the parenchyma cells surrounding the vessels (Rioux and Ouellette 1991, Tippett and Shigo 1981). The DED fungi gain access to

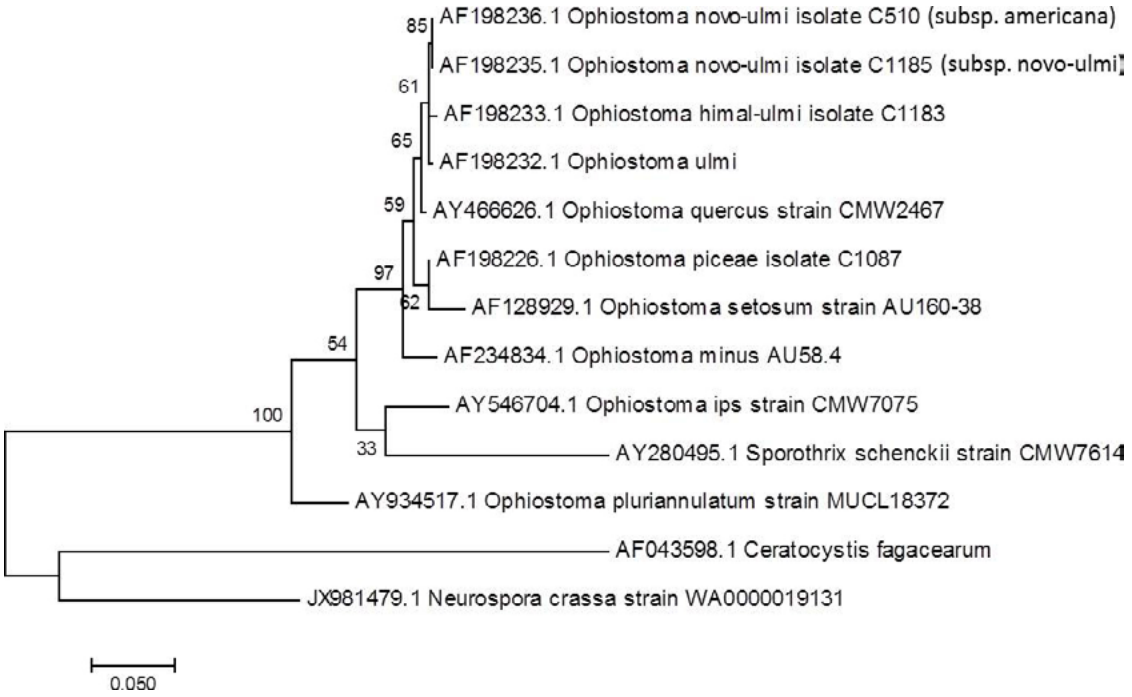

Figure 1.—Phylogenetic relationships between the Dutch elm disease (DED) fungi *Ophiostoma ulmi*, *O. novo-ulmi,* and *O. himal-ulmi*, and other Ascomycete species.

Internal Transcribed Spacer 1 (ITS1) sequences were downloaded from the National Center for Biotechnology Information (NCBI) database. Sequences were aligned and concatenated with BioEdit 7.2.5 software (Hall 1999). A maximum-likelihood tree was constructed with Mega6 with 1000 bootstraps (Tamura et al. 2013). Bootstrap support is indicated on the nodes. The DED pathogens are closely related to sapstaining *Ophiostoma* species, including *O. quercus*, and to the human pathogen *Sporothrix schenckii*. *Ceratocystis fagacearum*, the oak wilt pathogen, is a distant relative. Figure created by Martha Nigg. More exhaustive phylogenetic trees including the DED fungi and other Ophiostomatales can be found in De Beer et al. (2016) and Ploetz et al. (2013).

the xylem of healthy elms through their association with elm bark beetles in the genera *Scolytus* and *Hylurgopinus* (Fig. 2). In the field, the DED pathogens appear to be specific to species in the genus *Ulmus*. Results from controlled inoculations of *Prunus pensylvanica* with *O. novo-ulmi*, however, have shown that the potential host range of the DED fungi extends beyond the range of its vectors (Rioux and Ouellette 1989).

Based on the extensive mortality incurred by elms worldwide in the last 100 years, it is obvious that the DED fungi are very efficient pathogens. Not surprisingly, there have been several studies devoted to the identification of biological traits that contribute to parasitic fitness (*sensu* Andrivon 1993). Most studies have focused on pathogenicity and several mechanisms have been proposed over the years to explain how the DED fungi kill elms. These include the release of toxins, cell-wall degrading enzymes, inhibitors of elm resistance mechanisms, or a combination of these. None of these mechanisms, however, has been demonstrated conclusively to be a main contributor to pathogenesis. For example, a small secreted hydrophobin, designated cerato-ulmin (CU), was once described as a wilt-inducing toxin responsible for the high virulence of *O. novo-ulmi* (Stevenson et al. 1979, Takai et al. 1983). It is not considered a pathogenicity factor anymore based on the recovery of virulent *O. novo-ulmi* mutants lacking the ability to produce CU (Bowden et al. 1996, Brasier et al. 1995).

The DED fungi exhibit yeast-mycelium dimorphism, a feature found in several fungal pathogens of plants (e.g., *Ustilago maydis*, *Verticillium albo-atrum*) and animals (e.g., *Candida albicans*, *Histoplasma capsulatum,* and the Ophiostomatale species *Sporothrix schenckii*). Several environmental factors will prompt the DED fungi to switch from one growth form to the other

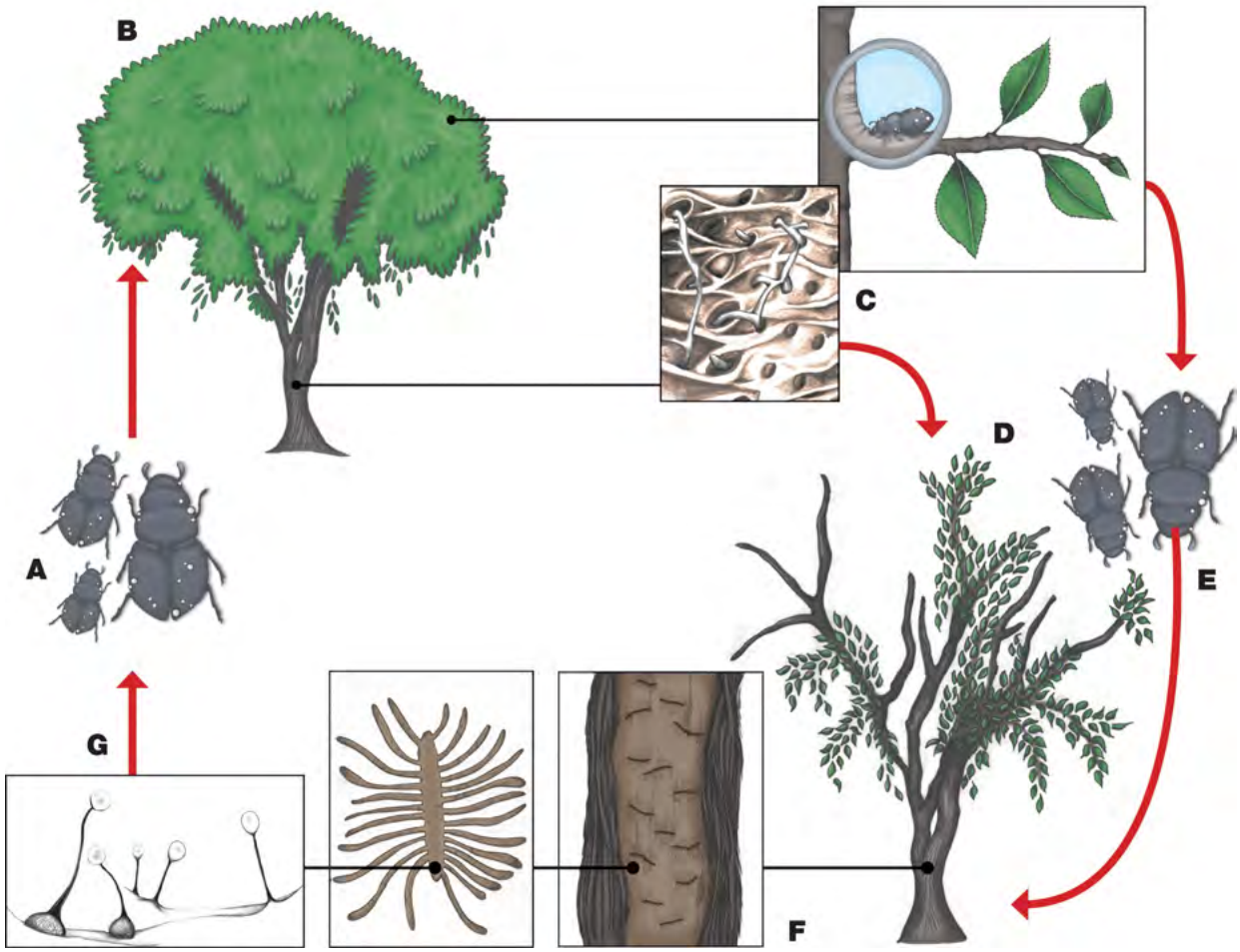

Figure 2.—Dutch elm disease (DED) cycle.

Young elm bark beetles carrying spores of DED fungi (A) and feeding on healthy trees (B) provide the pathogens with access to water-conducting vessels within the xylem. The DED fungi produce both yeast-like spores and mycelium when invading the vascular system (C). Infection of susceptible elms results in wilting and eventually death (D). Virgin female bark beetles looking for suitable breeding sites are attracted by trees that have been killed or weakened by DED. Many of these beetles carry spores of DED fungi (E), which will readily colonize galleries in which females have oviposited after mating (F). Within the galleries, the DED fungi produce reproductive structures (G) including asexual synnemata and sexual perithecia. Spores produced by both structures are embedded in a drop of sticky mucilage and will easily attach to the exoskeleton of young elm bark beetle adults (A) emerging from the galleries. While candidate genes associated with pathogenicity, yeast-mycelium dimorphism, and formation of fruiting bodies have been identified, the molecular bases of the host-pathogen interaction leading to DED remain unknown. Figure created by Marilou Desharnais and reprinted from Comeau et al. 2015, with permission.

(Berrocal et al. 2012, Kulkarni and Nickerson 1981, Naruzawa and Bernier 2014, Naruzawa et al. 2016, Wedge et al. 2016). Results by Richards (1994) suggest that the ability to transition between the mycelium and yeast phases is important for pathogenicity of *O. novo-ulmi*. Ongoing transcriptomic analyses of yeast-mycelium dimorphism in *O. novo-ulmi* have shown that at least 10 percent of the nuclear genes undergo a significant shift in transcription during the transition *in vitro* (Nigg et al. 2015) and that the remodeling of the transcriptome occurs early in the transition (Nigg and Bernier 2016). Furthermore, several genes that may be key regulators of dimorphism, and possibly pathogenicity, have been identified as good candidates for further studies.

The interaction between the DED fungi and elm bark beetles is likely another key component of overall fitness. In contrast to other vector-fungus associations in which the two organisms live in symbiosis, elm bark beetles and DED fungi seem to enjoy a more unidirectional interaction

from which the fungus is the main benefactor. It has been estimated that an individual elm bark beetle may carry up to 350,000 spores on its exoskeleton (Webber 1990). Cerato-ulmin appears to be a key molecule in the interaction between elm bark beetles and *Ophiostoma* spp., as mutants of *O. novo-ulmi* impaired in CU production were shown to be less efficient than wild-type isolates in attaching to insects (Temple et al. 1997). McLeod et al. (2005) suggested that the DED fungi manipulated elms to make them more attractive to elm bark beetles. This hypothesis, if verified, would put the elm bark beetle-*Ophiostoma* interaction in a context very different from the unidirectional view mentioned above.

Dutch elm disease fungi survive as saprophytes in dead or dying elms. This part of the fungal life cycle has received limited attention compared to the pathogenic phase, even though it plays an important role in both the short- and long-term evolution of the disease. Breeding galleries that are dug by beetles under the bark are colonized by mycelium of DED fungi. As reviewed by Santini and Faccoli (2015), fungal isolates found in these galleries may originate either from the pathogenic phase (through movement of the pathogen from the xylem towards the phloem) or from new inoculations by female beetles when they colonize the bark. Not surprisingly, mosaics of genotypes have been reported in populations of DED isolates recovered from inner elm bark (Webber et al. 1986). When they colonize elm beetle galleries, the DED fungi undergo another important developmental change as they produce synnemata, asexual structures bearing synnematal spores. Sexually compatible individuals within the same gallery may mate and produce perithecia containing recombinant ascospores. Both synnemata and perithecia exhibit features that favor acquisition of the pathogen by elm bark beetles, as masses of synnematal spores and ascospores are embedded in droplets of sticky mucilage. Furthermore, the synnema head fluid and the ostiolar hairs on perithecia of *O. novo-ulmi* have been shown to contain high amounts of CU (Svircev et al. 1988, Takai et al. 1980). Fungal genotypes able to dominate the populations occurring within breeding galleries are likely to be preferentially inoculated to healthy elms by young beetles emerging from the galleries during maturation feeding time (Fig. 2).

Origin of the DED Fungi

Based on pollen counts, the first documented case of massive decline of elm populations occurred in western Europe during the early Neolithic, around 5,500 calibrated years before present (Batchelor et al. 2014). This decline has been attributed by some authors to interactions among causal factors including paludification, deforestation for agriculture, and disease. Although macrofossils of two known vectors of DED fungi, *S. scolytus* and *S. multistriatus*, have been found at some sites, there is no evidence that these insects had already acquired the DED pathogens at the time. Therefore, the Dutch elm disease pandemics that have been developing since the early part of the 20th century in Eurasia and North America are, unless new evidence arises to the contrary, a "modern" disease that resulted from the introduction of exotic pathogens. It was widely believed that *O. ulmi*, responsible for the first pandemic, originated from China (Horsfall and Cowling 1978) but the fungus was never found in that country (e.g., Brasier 1990). A more recent hypothesis is that *O. ulmi* originated from Japan (Masuya et al. 2010). Likewise, the geographic origin of *O. novo-ulmi,* responsible for the current pandemic, remains the object of much speculation. Subspecies *novo-ulmi* and *americana* are thought to have originated in central Europe and around the North American Great Lakes, respectively (Brasier 1996). The identity and geographic origin of their common ancestor, however, is unknown. Based on available genetic and genomic data (Comeau et al. 2015, Khoshraftar et al. 2013), it is nevertheless clear that *O. novo-ulmi* is not a mutant that arose recently from *O. ulmi* and that the two species in fact diverged some time ago (Fig. 1). The third DED pathogen, *O. himal-ulmi*, is, so far, reported only on the Indian side of the Himalayas (Brasier and Mehrotra 1995). The full extent of its geographic range in Asia is unknown.

Shifts That Have Happened in the DED Fungi and Their Consequences

The 100 years of DED pandemics have been marked by biological events with dire consequences for elms and elm resistance breeding programs. One such event was the advent of *O. novo-ulmi*, a highly fit and aggressive pathogen able to attack and kill native European elm species, as well as early releases of elm cultivars that were tolerant to *O. ulmi*. Thus, years of breeding efforts were lost within a short timespan because of an event that nobody had foreseen. The high fitness of *O. novo-ulmi* has allowed it to rapidly displace the less competitive *O. ulmi* from most areas impacted by the disease. Another significant event, however, took place while both species co-occurred in the same geographic areas: *O. novo-ulmi* acquired "useful" alleles from *O. ulmi* through interspecific hybridization. One such acquisition is the *MAT1-1* mating-type allele, which was not detected in initial surveys of *O. novo-ulmi* (Brasier 1988, Paoletti et al. 2006). Therefore, reproduction in earlier-day populations of *O. novo-ulmi* was essentially clonal. This is no longer the case, since the *MAT1-1* and *MAT1-2* alleles both occur in current populations of the fungus and allow sexual recombination to take place. A third event with biological importance was the rise of two genetically distinct subspecies within *O. novo-ulmi*, followed by the occurrence of sexual recombination between the subspecies in areas of Europe where they are both present. As documented by Brasier and Kirk (2010), many of the resulting hybrids exhibit high pathogenic fitness.

Will There Be Other Important Shifts in the DED Fungus Species and Populations?

The evolution of fungal populations is dependent on their size, occurrence of mutations that affect the phenotype, and frequency of asexual and sexual reproduction (Zeyl 2009). Mutations typically result from errors in DNA repair mechanisms during the normal life cycle or following external stresses (Rattray and Strathern 2003). Mutations can also result from the insertion and movement of foreign DNA such as transposons (Daboussi and Capy 2003). Many biological features of the DED fungi should favor the accumulation and combination of mutations that may contribute to new genotypes, including some with improved parasitic fitness. Large populations of synnematal spores produced in bark beetle galleries are expected to contain a proportion of spontaneous mutants with altered phenotypes which can be recombined through sexual crosses to yield recombinants with higher fitness. Some novel phenotypes may have been caused by insertion or excision of mobile elements such as the OPHIO DNA transposons (Bouvet et al. 2007, 2008) and SWING retrotransposons (Comeau et al. 2015). With the rapid demise of *O. ulmi*, interspecific hybridization events are less likely to occur. However, introgression of hybrids to *O. novo-ulmi* could yield highly fit individuals. For example, the progeny from a laboratory cross between highly virulent *O. novo-ulmi* strain H327 and less virulent introgressant strain AST 27 (Et-Touil et al. 1999) included a few highly virulent individuals with an extended growth temperature range (Et-Touil 2000).

In nature, *O. ulmi* and *O. novo-ulmi* are geographically isolated from *O. himal-ulmi*. Results from laboratory crosses led Brasier and Mehrotra (1995) to conclude that there was also strong (but incomplete) reproductive isolation between *O. himal-ulmi* and the other two DED fungi. When crosses occurred, F_1 progeny were reported to display strong negative interactions for mycelial growth rate compared to parental growth rate means (Brasier and Mehrotra 1995). To our knowledge, however, other components of parasitic fitness such as pathogenicity and virulence, were not examined by these authors. Furthermore, all *O. himal-ulmi* strains tested originated from the same location in northern Himachal Pradesh. Therefore, one cannot

rule out the possibility that highly virulent and reasonably fit hybrids might arise from some encounters between *O. himal-ulmi* and the other DED fungi.

Although other known members of the '*Ophiostoma piceae-Ophiostoma ulmi*' complex are considered to be saprophytes, the possibility that one of them might hybridize with any of the DED fungi is a relevant issue. According to Brasier (1993), there is strong reproductive isolation between the DED pathogens (*O. ulmi* and *O. novo-ulmi*) and the saprobes *O. piceae* and *O. quercus*. Recent results from various interspecific pairings carried out in the author's laboratory support this conclusion, with the caveat that the number of isolates subjected to interspecific pairings was relatively modest. However, one pairing between *O. himal-ulmi* and *O. quercus* yielded large numbers of viable offspring (Bernier, unpublished results). The ability of *O. himal-ulmi* and *O. quercus* to mate is not that surprising given their relative phylogenetic proximity (Fig. 1). Although results from laboratory pairings must be interpreted with caution, the above results suggest an additional way for DED fungi to acquire new traits through interspecific hybridization. Upcoming analyses of *O. himal-ulmi* × *O. quercus* cross will shed light on the parasitic fitness of the F_1 progeny and potential emergence of highly fit individuals within the progeny.

The saprobe *O. quercus* probably deserves renewed attention. Del Sorbo et al. (2000) reported that they had recovered individuals that were pathogenic to elms among a collection of *O. quercus* mutants transformed with the *O. novo-ulmi* CU gene. The authors' conclusion was that CU was a pathogenicity factor, thus contradicting conclusions to the contrary by Bowden et al. (1996), Brasier et al. (1995), and Temple et al. (1997). However, not all *O. quercus* CU-producing transformants were pathogenic to elms. An alternative explanation for the occurrence of pathogenic *O. quercus* individuals could be that random integration of the CU gene in their genome had inactivated effector genes encoding molecules that trigger elm resistance against wild-type *O. quercus*. In any case, the results obtained by Del Sorbo and colleagues (2000) suggest that a very thin line separates *O. quercus* from becoming a pathogen of elms and reinforce previous speculation that the DED fungi might have originated from a phylogenetically close sapstaining species of *Ophiostoma* (Brasier 1990).

As mentioned previously, results from inoculations to nonhost species have confirmed that *O. novo-ulmi* can successfully attack at least one species (*Prunus pensylvanica*) not related to the genus *Ulmus* (Rioux and Ouellette 1989). This suggests that the specificity of the DED fungi toward elms observed in nature results in good part from their association with elm bark beetles. Therefore, if *O. novo-ulmi* were acquired by an insect vector that could inoculate the fungus to *P. pensylvanica*, this could result in a successful host jump for the pathogen, as already proposed by Brasier (1990) who pointed out that *S. scolytus, S. laevis,* and *S. pigmaeus* did not feed only on elms. One may therefore wonder if new diseases of elms could also occur if another ophiostomatoid (e.g., the oak wilt fungus *Ceratocystis fagacearum*) or non-ophiostomatoid pathogen were acquired by elm bark beetles through feeding or breeding on a different host. To the author's knowledge, few beetle-associated fungal species outside of the DED fungi have been tested on North American elms in controlled inoculation trials, with the exception of the sapstaining species *Ceratocystis* (now *Endoconidiophora*) *resinifera* (Morin et al. 2007). Therefore, it would seem a good idea to carry out controlled inoculations of *U. americana* with selected pathogens that are currently thought to be specific to other host species.

Genome-wide Investigations of Parasitic Fitness in the DED Fungi

Our knowledge of the biology and evolution of the DED fungi stems mostly from small-scale studies of specific biological traits and, in some cases, of their genetic and molecular determinants. These studies have been facilitated by the relative ease with which the DED fungi can be grown and manipulated in the laboratory. More recently, post-Sanger sequencing technologies have been used to explore the genomes and transcriptomes of *O. ulmi* strain W9 (Khoshraftar et al. 2013) and *O. novo-ulmi* subsp. *novo-ulmi* strain H327 (Comeau et al. 2015, Forgetta et al. 2013, Hintz et al. 2011, Jacobi et al. 2010, Nigg and Bernier 2016, Nigg et al. 2015).

The nuclear genomes of *O. ulmi* and *O. novo-ulmi* are similar in size (*ca* 32Mb) and gene content (8639 and 8640 predicted genes, respectively). Not surprisingly, these genomes contain a wide repertoire of genes that encode putative pathogenicity and virulence factors, enzymes known to degrade plant cell wall constituents, oxidative enzymes, and secondary metabolites (Comeau et al. 2015, Khoshraftar et al. 2013). When the genome of *O. novo-ulmi* is compared with those of other Ascomycete species, however, interesting trends appear (Table 1). The genome of *O. novo-ulmi* is smaller and contains fewer genes. In the case of genes encoding cytochrome P450 oxydases (CYP450s), which may be involved in the synthesis of toxins and detoxification of plant resistance molecules, it is noteworthy that *O. novo-ulmi* possesses a smaller and less diversified repertoire than other vascular plant pathogens such as *Fusarium oxysporum*, *Verticillium dahlia,* and *V. albo-atrum* (Moktali 2013). In their genomic analysis of *V. dahliae* and *V. albo-atrum*, Klosterman et al. (2011) identified the expansion in genes encoding carbohydrate-active enzymes (CAZymes) as a feature of vascular wilt fungi. Once again, the genome of *O. novo-ulmi* does not fit this model, as it contains a relatively modest number of CAZyme-encoding genes. Comeau et al. (2015) suggested this may reflect the fact that the association of DED fungi with elm bark beetles gives them direct access to the elm vascular system, whereas *F. oxysporum* and *Verticillium* spp. have to penetrate outer layers of protective plant tissue. Further validation of this hypothesis must await the public release and comparison of annotated genomes from more fungal pathogens, including species that are associated with vectors and species that are not.

Once a genome has been sequenced, the next step is to validate the actual function and contribution of candidate genes identified *in silico*. Genome-wide gene profiling by RNA-Seq is a powerful tool for this purpose and has been used successfully in ongoing investigations of yeast-mycelium dimorphism in *O. novo-ulmi*. Based on RNA-Seq data, it is clear that a substantial (10 percent) portion of *O. novo-ulmi* genes are differentially expressed between the two growth phases (Nigg et al. 2015) and that significant changes in the transcriptome occur as early as 2 hours after the switch from yeast to mycelium under defined laboratory conditions (Nigg and Bernier 2016). Since yeast-mycelium dimorphism in the DED fungi can be induced by a variety of physical and chemical stimuli, transcriptomic analyses run under different sets of conditions may provide insight into different metabolic pathways used by the pathogen to transition between the two growth forms. Other biological traits of the DED fungi are amenable to RNA-Seq analyses, both *in vitro* and *in planta*. Although the recovery of fungal transcriptomes from inoculated elms is technically challenging due to the overrepresentation of plant messenger RNA (Aoun et al. 2010, Perdiguero et al. 2015), it can be achieved, as reported by Sherif and colleagues during the 2016 Elm restoration workshop. This is an important development for the eventual identification of fungal transcripts (and ultimately molecules) that are produced when the pathogen interacts with its host.

Table 1.—Partial genomic comparisons between *Ophiostoma novo-ulmi* and other pathogens and saprobes belonging to the Sordariomycetes (Ascomyceta). Compiled from data from Comeau et al. (2015), Klosterman et al. (2011), Lah et al. (2013), and Moktali (2013).

	Order							
	Ophiostomatales[a]		Hypocreales	*Incertae sedis*	Magnaporthales	Sordariales		
	Ophiostoma novo-ulmi	*Grosmannia clavigera*	*Fusarium oxysporum f.sp. lycopersici*	*Verticillium dahliae*	*Magnaporthe oryzae*	*Chaetomium globosum*	*Neurospora crassa*	*Podospora anserine*
Lifestyle	Pathogen	Pathogen	Pathogen, saprobe	Pathogen	Pathogen	Saprobe	Saprobe	Saprobe
Genome size (Mb)	31.8	29.8	61.5	33.8	41.0	34.9	41.0	33.5
Total nb genes	8,640	8,314	17,735	10,535	11,074	11,124	9,734	10,545
CYP450[b]	48	54	169	69	135	92	43	115
Nb families	39	44	96	55	77	69	39	72
CAZymes[c]								
GH	165	146	367	265	262	233	172	205
PL	2	4	27	36	5	15	4	7
CE	20	7	50	50	52	39	21	39
CBM	35	26	91	82	120	87	43	96
GT	77	82	126	97	102	93	76	87
AA	12	4	33	29	30	45	17	38
Total CAZyme	**311**	**269**	**694**	**559**	**571**	**512**	**333**	**472**
% CAZyme[d]	3.60	3.24	3.91	5.31	5.16	4.60	3.42	4.48

[a]*Ophiostoma ulmi* is not incuded in the table since its CAZYme genes have not been annotated. The other member of the Ophiostomatales listed in the table, *Grosmannia clavigera*, is associated with the current mountain pine beetle (*Dendroctonus ponderosae*) outbreak, which has killed millions of ponderosa (*Pinus ponderosae*) and lodgepole (*P.contorta*) pines in western North America.

[b]CYP450: genes encoding cytochromes P450 oxydases.

[c]CAZYmes: genes encoding carbohydrate-active Enzymes. The following CAZYme families are recognized: glycoside hydrolases (GH), polysaccharide lyases (PL), carbohydrate esterases (CE), carbohydrate-binding modules (CBM), glycosyltransferases (GT), auxiliary activities (AA).

[d] Percentage CAZyme: percentage of CAZyme-encoding genes in the genome.

Full validation of gene function, however, requires the recovery and analysis of null mutants in which targeted genes have been inactivated. Nowadays, this is achieved through insertional mutagenesis and the resulting null mutants are also known as knockout mutants. Mutants for genes encoding cerato-ulmin (Bowden et al. 1996), endopolygalacturonase (Temple et al. 2009) and cyclooxygenase cox1 (Naruzawa 2015) have been obtained from *O. novo-ulmi* but the overall efficiency of recovery by standard procedures remains extremely low. Systematic inactivation of genes and analysis of the resulting phenotypes in knockout mutants must await the development of more efficient procedures for the mutagenesis of targeted genes. In the meantime, gene knockdown by RNA interference remains the best alternative for the DED fungi (Carneiro et al. 2010, 2013).

Full-scale "omic" investigations of DED fungi (and their saprophytic relatives), however, require financial means that were not available for previous projects. Fortunately, a new project, BioSAFE, launched in late 2016 and funded by Genome Canada, Genome British Columbia, and Genome Quebec will provide financial resources for a more thorough investigation of the DED fungi. The project aims at using next-generation sequencing approaches for understanding the development of the two successive DED pandemics through genomic profiling of fungal populations, identifying genes and markers associated with fitness and outbreak-associated biological traits, and developing biosurveillance tools. It is expected that large-scale, genome-wide investigations will help reconstruct the evolutionary origin of the DED pathogens, understand the dynamics underlying the replacement of *O. ulmi* by *O. novo-ulmi*, and provide plausible scenarios for the continuing evolution of DED fungi. Tools for achieving these goals will include *de novo* sequencing and comparative analysis of many *Ophiostoma* genomes (including pathogens and saprobes), genomic and phenotypic analysis of progeny from controlled genetic crosses, as well as development of protocols for efficient induction and recovery of knockout mutants for targeted genes.

Conclusion

The face of DED research in North America has changed dramatically since the 1981 DED symposium and workshop held in Winnipeg (Kondo et al. 1982). Unfortunately, the last decades have seen a decline in the number of scientists and level of funding, and this decline has been particularly evident in government agencies such as the Canadian Forest Service. However, several programs have been maintained in academia and government agencies, and new research groups have become involved, both in the United States and Canada. Contrary to the notion that North American elm species might become extinct (or have already been eradicated, as one can sometimes read in the popular press), survivor elms have been identified and are being integrated into disease resistance breeding programs. As more survivor trees are added to the program, one can envision that a comprehensive catalogue of DED resistant North American elms will become available. The identification, by fungal geneticists, of molecular determinants of pathogenicity in DED fungi should, in turn, help tree geneticists target genes contributing to resistance. Although this is a long-term endeavor, combining desirable alleles from several genes into single elm genotypes would be expected to promote longer-term resistance to DED. It will also be important to keep monitoring the DED pathogens closely, and possibly other fungal species that do not currently cause problems but may evolve into pathogens of elms, in order to make sure that the current efforts to restore North American elms are not annihilated by a new pandemic.

Acknowledgments

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Literature Cited

Andrivon, D. 1993. **Nomenclature for pathogenicity and virulence: The need for precision.** Phytopathology. 83: 889-890.

Aoun, M.; Jacobi, V.; Boyle, B; Bernier, L. 2010. **Identification and monitoring of *Ulmus americana* transcripts during in vitro interactions with the Dutch elm disease pathogen *Ophiostoma novo-ulmi*.** Physiological and Molecular Plant Pathology. 74: 254-266.

Batchelor, C.R.; Branch, N.P.; Allison, E.A.; Austin, P.A.; Bishop, B. [et al.] 2014. **The timing and causes of the Neolithic elm decline: New evidence from the Lower Thames Valley (London, UK).** Environmental Archaeology. 19: 263-290.

Bernier, L. 2016. **Genome-wide analysis of parasitic fitness traits in a non-model tree pathogen.** Canadian Journal of Plant Pathology. 38: 153-163

Bernier, L.; Aoun, M.; Bouvet, G.F.; Comeau, A.; Dufour, J. [et al.]. 2015. **Genomics of the Dutch elm disease pathosystem: are we there yet?** iForest. 8: 149-157.

Berrocal, A.; Navarrete, J.; Oviedo, C.; Nickerson, K.W. 2012. **Quorum sensing activity in *Ophiostoma ulmi* : effects of fusel oils and branched chain amino acids on yeast-mycelial dimorphism.** Journal of Applied Microbiology. 113: 126-134.

Bouvet, G.F.; Jacobi, V.; Bernier, L. 2007. **Characterization of three DNA transposons in the Dutch elm disease fungi and evidence of repeat induced point (RIP) mutations.** Fungal Genetics and Biology. 44: 430-443.

Bouvet, G.F.; Jacobi, V.; Plourde, K.V.; Bernier, L. 2008. **Stress-induced mobility of OPHIO1 and OPHIO2, DNA transposons of the Dutch elm disease fungi.** Fungal Genetics and Biology. 45: 565-578.

Bowden, C.G.; Smalley, E; Guries, R.P.; Hubbes, M.; Temple, B; Horgen, P.A. 1996. **Lack of association between cerato-ulmin production and virulence in *Ophiostoma novo-ulmi*.** Molecular Plant-Microbe Interactions. 9: 556-564.

Brasier, C.M. 1983. **The future of Dutch elm disease in Europe.** In: Burdekin, D.A., ed. Research on Dutch elm disease in Europe. Proceedings of the European economic community research seminar; 1982 March 30–April 1; Guernsey, Channel Islands. Forestry Commission Bulletin. 60: 96-104.

Brasier, C.M. 1988. **Rapid changes in genetic structure of epidemic populations of *Ophiostoma ulmi*.** Nature. 332: 538-541.

Brasier, C.M.1990. **China and the origins of Dutch elm disease: an appraisal.** Plant Pathology. 39: 5-16.

Brasier, C.M. 1991. ***Ophiostoma novo-ulmi* sp. *nov*., causative agent of current Dutch elm disease pandemics.** Mycopathologia. 115: 151-161.

Brasier, C.M. 1993. **The genetic system as a fungal taxonomic tool: gene flow, molecular variation and sibling species in the '*Ophiostoma piceae* -*Ophiostoma ulmi*' complex and its taxonomic and ecological significance.** In: Wingfield, M.J.; Seifert, K.; Webber, J.F., eds. *Ophiostoma* and *Ceratocystis:* taxonomy, biology and pathology. St. Paul, MN: American Phytopathological Society: 77-92.

Brasier, C.M. 1996. **Low genetic diversity of the *Ophiostoma novo-ulmi* population in North America.** Mycologia. 88: 951-964.

Brasier, C.M.; Kirk, S.A. 2001. **Designation of the EAN and NAN races of *Ophiostoma novo-ulmi* as subspecies.** Mycological Research. 105: 547-554.

Brasier, C.M.; Kirk, SA. 2010. **Rapid emergence of hybrids between the two subspecies of *Ophiostoma novo-ulmi* with a high level of pathogenic fitness.** Plant Pathology. 59: 186-199.

Brasier, C.M.; Kirk, S.A.; Tegli, S. 1995. **Naturally occurring non cerato-ulmin producing mutants of *Ophiostoma novo-ulmi* are pathogenic but lack aerial mycelium.** Mycological Research. 99: 436-440.

Brasier, C.M.; Mehrotra, M.D. 1995. ***Ophiostoma himal-ulmi* sp. *nov*., a new species of Dutch elm disease fungus endemic to the Himalayas.** Mycological Research. 99: 205-215.

Carneiro, J.S.; de la Bastide, P.Y.; Chabot, M.; Lerch, L.; Hintz, W.E. 2010. **Suppression of polygalacturonase gene expression in the phytopathogenic fungus *Ophiostoma novo-ulmi* by RNA interference.** Fungal Genetics and Biology. 47: 399-405.

Carneiro, J.S.; de la Bastide, P.Y.; Hintz, W.E. 2013. **Regulated gene silencing in the fungal pathogen *Ophiostoma novo-ulmi*.** Physiological and Molecular Plant Pathology. 82: 28-34.

Comeau, A.M.; Dufour, J.; Bouvet, G.F.; Nigg, M.; Jacobi, V. [et al.]. 2015. **Functional annotation of the *Ophiostoma novo-ulmi* genome: insights into the phytopathogenicity of the fungal agent of Dutch elm disease.** Genome Biology and Evolution. 7: 410-430.

Daboussi,M.-J.; Capy, P. 2003. **Transposable elements in filamentous fungi.** Annual Review of Microbiology. 57: 275-99.

Del Sorbo, G.; Scala, F.; Parrella, G.; Lorito, M.; Comparini, C.[et al.]. 2000. **Functional expression of the gene cu, encoding the phytotoxic hydrophobin cerato-ulmin, enables *Ophiostoma quercus*, a nonpathogen on elm, to cause symptoms of Dutch elm disease.** Molecular Plant-Microbe Interactions. 13: 43-53.

De Beer, Z.W.; Duong, T.A.; Wingfield, M.J. 2016. **The divorce of *Sporothrix* and *Ophiostoma*: solution to a problematic relationship.** Studies in Mycology. 83: 165-191.

Et-Touil, A. 2000. **Étude génétique, biochimique et physiologique de la pathogénécité chez *Ophiostoma novo-ulmi* [Genetic, biochemical and physiological studies of *Ophiostoma novo-ulmi*].** Quebec City, QC: Laval University. Ph.D. dissertation.

Et-Touil, A.; Brasier, C.M.; Bernier, L. 1999. **Localization of a pathogenicity gene in *Ophiostoma novo-ulmi* and evidence that it may be introgressed from *O. ulmi*.** Molecular Plant-Microbe Interactions. 12: 6-15.

Forgetta, V.; Leveque, G.; Dias, J.; Grove, D.; Lyons, R., Jr. [et al.]. 2013. **Comparison of multiple genome sequencing centers and analysis of the Dutch elm disease fungus genome using the Roche/454 GS-FLX Titanium System.** Journal of Biomolecular Techniques. 24: 39-49.

Ganley, R.J.; Bulman, L.S. 2016. **Dutch elm disease in New Zealand: impacts from eradication and management programmes.** Plant Pathology. 65: 1047-1055.

Hall, T. 1999. **BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.** Nucleic Acids Symposium Series. 41: 95-98.

Hintz, W.; Pinchback, M.; de la Bastide, P.; Burgess, S.; Jacobi, V. [et al.]. 2011. **Functional categorization of unique expressed sequence tags obtained from the yeastlike growth phase of the elm pathogen *Ophiostoma novo-ulmi*.** BMC Genomics. 12: 431.

Holmes, F.W.; Heybroek, H.M., eds. 1990. **Dutch elm disease: The early papers: Selected works of seven Dutch women scientists.** St. Paul, MN: American Phytopathological Society Press. 154 p.

Horsfall, J.G.; Cowling, E.B. 1978. **Some epidemics man has known.** In: Horsfall, J.G.; Cowling, E.B., eds. Plant disease, an advanced treatise. New York, NY: Academic Press: 17-32.

Houston, D.R. 1991. **Changes in aggressive and nonaggressive subgroups of *Ophiostoma ulmi* within two populations of American elm in New England.** Plant Disease. 75: 720-722.

Jacobi, V.; Dufour, J.; Bouvet, G.F.; Aoun, M.; Bernier, L. 2010. **Identification of transcripts up-regulated in asexual and sexual fruiting bodies of the Dutch elm disease pathogen *Ophiostoma novo-ulmi*.** Canadian Journal of Microbiology. 56: 697-705.

Khoshraftar, S.; Hung, S.; Khan, S.; Gong, Y.; Tyagi, V. [et al.]. 2013. **Sequencing and annotation of the *Ophiostoma ulmi* genome.** BMC Genomics. 14: 162.

Klosterman, S.J.; Subbarao, K.V.; Kang, S.; Veronese, P.; Gold, S.E. [et al.]. 2011. **Comparative genomics yields insights into niche adaptation of plant vascular wilt pathogens.** PLoS Pathogens. 7: e1002137.

Kondo, E.S.; Hiratsuka, Y; Denyer, W.B.G., eds. 1982. **Proceedings of the Dutch elm disease symposium and workshop**; 1981 October 5-9; Winnipeg, Manitoba, CA. Winnipeg, Manitoba, CA: Manitoba Department of Natural Resources. [pages unknown].

Kulkarni, R.K.; Nickerson, K.W. 1981. **Nutritional control of dimorphism in *Ceratocystis ulmi*.** Experimental Mycology. 5:148-154.

Masuya, H.; Brasier, C.; Ichihara, Y.; Kubonoa, T.; Kanzaki, N. 2010. **First report of the Dutch elm disease pathogens *Ophiostoma ulmi* and *O. novo-ulmi* in Japan.** Plant Pathology. 59: 805.

McLeod, G.; Gries, R.; von Reus, S.H.; Rahe, J.E.; McIntosh, R.; Konig, W.A.; Gries, G. 2005. **The pathogen causing Dutch elm disease makes host trees attract insect vectors.** Proceedings of the Royal Society B-Biological Sciences. 272: 2499-2503.

Moktali, V. 2013. **Niche adaptation in fungi.** University Park, PA: Pennsylvania State University. Ph.D. dissertation.

Morin, C.; Couturier, S.; Bernier, L. 2007. **Pathogenicity of wild-type and albino strains of the fungus *Ceratocystis resinifera*, a potential biocontrol agent against bluestain.** Canadian Journal of Forest Research. 37: 919-930.

Naruzawa, E.S. 2015. **Bases moléculaires du dimorphisme levure-mycélium chez le champignon phytopathogène *Ophiostoma novo-ulmi* [Molecular bases of yeast-mycelium dimorphism in the plant pathogenic fungus *Ophiostoma novo-ulmi*].** Quebec City, QC: Laval University. Ph.D. dissertation.

Naruzawa, E.S.; Bernier, L. 2014. **Control of yeast-mycelium dimorphism in vitro in Dutch elm disease fungi by manipulation of specific external stimuli.** Fungal Biology. 118: 872-884.

Naruzawa, E.S.; Malagnac, F.; Bernier, L. 2016. **Effect of linoleic acid on reproduction and yeast-mycelium dimorphism in the Dutch elm disease pathogens.** Botany. 94: 31-39.

Nigg, M.; Bernier, L. 2016. **From yeast to hypha: defining transcriptomic signatures of the morphological switch in the dimorphic pathogen fungus *Ophiostoma novo-ulmi*.** BMC Genomics. 17: 920.

Nigg, M.; Laroche, J.; Landry, C.R.; Bernier, L. 2015. **RNAseq analysis highlights specific transcriptome signatures of yeast and mycelial growth phases in the Dutch elm disease fungus *Ophiostoma novo-ulmi*.** G3: Genes |Genomes |Genetics. 5: 2487-2495.

Paoletti, M; Buck, K.W.; Brasier, C.M. 2006. **Selective acquisition of novel mating type and vegetative incompatibility genes via interspecies gene transfer in the globally invading eukaryote *Ophiostoma novo-ulmi*.** Molecular Ecology. 15: 249-262.

Perdiguero, P.; Venturas, M.; Cervera, M.T.; Gil, L.; Collada, C. 2015. **Massive sequencing of *Ulmus minor's* transcriptome provides new molecular tools for a genus under the constant threat of Dutch elm disease.** Frontiers in Plant Science. 6: 541.

Ploetz, R.C.; Hulcr, J.; Wingfield,M.J.; De Beer, Z.W. 2013. **Destructive tree disease associated with ambrosia and bark beetles; black swan events inplant pathology?** Plant Disease. 97: 856-872.

Rattray, A.J.; Strathern, J.N. 2003. **Error-prone DNA polymerases: when making a mistake is the only way to get ahead.** Annual Review of Genetics. 37: 31-66.

Richards, W.C. 1994. **Nonsporulation in the Dutch elm disease fungus *Ophiostoma ulmi*: evidence for control by a single nuclear gene.** Canadian Journal of Botany. 72: 461-467.

Rioux, D.; Ouellette, G.B. 1989. **Light microscope observations of histological changes induced by *Ophiostoma ulmi* in various nonhost trees and shrubs.** Canadian Journal of Botany. 67: 2335-2351.

Rioux, D.; Ouellette, G.B. 1991. **Barrier zone formation in host and nonhost trees inoculated with *Ophiostoma ulmi*.** I. **Anatomy and histochemistry.** Canadian Journal of Botany. 69: 2055-2073.

Santini, A; Faccoli, M. 2015. **Dutch elm disease and elm bark beetles: a century of association.** iForest. 8: 126-134.

Stevenson, K.J.; Slater, J.A.; Takai, S. 1979. **Cerato-ulmin, a wilting toxin of Dutch elm disease fungus.** Phytochemistry. 18: 235-238.

Svircev, A.M.; Jeng, R.S.; Hubbes, M. 1988. **Detection of cerato-ulmin on aggressive isolates of *Ophiostoma ulmi* by immunocytochemistry and scanning electron microscopy.** Phytopathology. 78: 322-327.

Takai, S.; Hiratsuka,Y.; Krywienczyk, Richards, W.C.; Davies, Y.P. 1980. **Evidence for the presence of the toxin cerato-ulmin in the synnema head fluid of *Ceratocystis ulmi*.** Canadian Journal of Botany. 58: 669-675.

Takai, S.; Richards, W.C.; Stevenson, K.J. 1983. **Evidence for the involvement of cerato-ulmin, the *Ceratocystis ulmi* toxin, in the development of Dutch elm disease.** Physiological Plant Pathology. 23: 275-280.

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. 2013. **MEGA6: Molecular evolutionary genetics analysis version 6.0.** Molecular Biology and Evolution. 30: 2725-2729.

Temple, B.; Bernier, L; Hintz, W.E. 2009. **Characterization of the polygalacturonase gene of the Dutch Elm Disease Pathogen *Ophiostoma novo-ulmi*.** New Zealand Journal of Forestry Science. 39: 29-37.

Temple, B.; Horgen, P.A.; Bernier, L.; Hintz, W.E. 1997. **Cerato-ulmin, a hydrophobin secreted by the causal agents of Dutch elm disease, is a parasitic fitness factor.** Fungal Genetics and Biology. 22: 39-53.

Tippett, J.T.; Shigo, A.L. 1981. **Barrier zone formation: a mechanism of tree defense against vascular pathogens.** IAWA [International Association of Wood Anatomists] Bulletin. 2: 163-168.

Tziros, G.T.; Nakopoulou, Z.G.; Perlerou, C.; Diamandis, S. 2017. **Current status of the Dutch elm disease pathogen populations affecting *Ulmus minor* in Greece.** Forest Pathology. 47(2): e12323.

Webber, J.F. 1990. **Relative effectiveness of *Scolytus scolytus, S. multistriatus,* and *S. kirschi* as vectors of Dutch elm disease.** European Journal of Forest Pathology. 20: 184-192.

Webber, J.F.; Brasier, C.M.; Mitchell, A.G. 1986. **The role of the saprophytic phase in Dutch elm disease.** In: Pegg. G.F.; Ayres, P.G., eds. Fungal infections of plants. Cambridge, UK: Cambridge University Press: 298-313.

Wedge, M.-E.; Naruzawa, E.S.; Nigg, M.; Bernier, L. 2016. **Diversity in yeast-mycelium dimorphism response of the Dutch elm disease pathogens: the inoculum size effect.** Canadian Journal of Microbiology. 62: 525-529.

Zeyl, C. 2009. **The role of sex in fungal evolution.** Current Opinion in Microbiology. 12: 592-598.

DUTCH ELM DISEASE: AN OVERVIEW OF THE BIOLOGY AND MANAGEMENT REGIMENS

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Much of the information on the Dutch elm disease (DED) topic was generated by a large group of dedicated scientists, in several different agencies, primarily in the United States and Europe, over the last century. My work on the fungicidal management is but a modest contribution to the whole. It goes without saying that much more work needs to be done to open up new fields, and to hone established doctrines and mechanisms on the disease.

Here, I present an overview of biology and management regimens for DED:

A. Prophylaxis (prevention) is always preferable to therapy (treatment of established disease). This, of course, is true for managing many problems of life, not just DED!

1. Scouting and early detection are crucial when implementing any management procedure. The one-liner I offer for this is the old Revolutionary War dictum: *eternal vigilance is the price of freedom*. Scouting must be expedited by well-trained scouts and on a regular (and very short interval) basis during the growing season when symptoms occur. In my many years working with cities and communities, I have learned that most people are "tree huggers," and will join together as a community in protecting their trees—a shared wealth. Educational programs led by an enthusiastic and knowledgeable promoter can greatly effect recruitment of volunteer help. The Master Gardener program is a wonderful vehicle through which this might be done.

2. Sanitation is likewise a "first must" when disease is detected and confirmed. Sanitation, when effectively implemented, reduces the inoculum density (pathogenic fungal mass) and insect vector populations. Communities, cities, or home owners who delay implementing early sanitation procedures will suffer the loss of nearby susceptible, healthy elms. **Early sanitation**, that is, tree removal at the first sighting of symptoms is the ultimate, and really, only viable choice, whereas **delayed sanitation** is often worthless in efficacy. DED management in cities practicing either system provide historically sharp contrasts in saving or losing elms.

3. Root graft severance is strongly indicated when susceptible, contiguous elms are growing within root graft distance of each other (25 to 50 feet), as the pathogen can move through the grafts from a diseased to a healthy tree. Once infection has been contracted via the roots, death is assured, and chemotherapy is useless.

4. Vector management has a major impact on lowering transmission of the pathogen from infected to healthy trees. Management in times past has been effective via crown sprays of insecticides, but because of environmental contamination, fewer compounds are legal for use in recent years. Of course, sanitation (above) helps to reduce or eliminate breeding sites of the bark beetle vector.

5. Fungicide infusion/injection is a most effective tool in preventing disease, and we might term it as a type of "immunization." Many compounds in past times have been tested *in vitro* and *in vivo*, and a few of them employed, but currently, Alamo® (propiconazole; Syngenta AG, Basel, Switzerland) and Arbotect® (thiabendazole

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hypophosphite; Syngenta AG, Basel Switzerland) are now commonly marketed and used to preclude initial infection, and to treat established disease. Propiconazole is more "tree friendly" (that is, less toxic) than thiabendazole, but propiconazole does not move from treated xylem (wood) to newly synthesized wood following injection as thiabendazole does. Further, infusion of larger volumes of propiconazole has been found to provide better (more uniform) translocation than concentrated concentrations applied via micro-injectors, when both are used at the recommended dosage rate based on diameter at breast height (d.b.h.). Even though propiconazole residues cannot be detected more than a year or so after infusion, protection from disease continues to occur. The late Mark Stennes and I theorize that a phytoalexin-like response is induced by the fungicide, thus lending disease resistance years following fungicide application.

6. The use of disease-resistant and/or disease-tolerant elms in new (or replacement) landscape designs is crucial where elms are chosen for the treescape. Many sites in times past were planted with one susceptible taxon, for example, the fully susceptible American elm, as many like the symmetry and the Gothic arch effect when elms line both sides of a street. In many situations as this, DED can proceed down the line from one tree to the next via root grafts or by close proximity of the crowns where fungal-contaminated beetles can go down the line from tree to tree. The one-liner that fits this is "symmetry can lead to cemetery." Another might be "variety is the spice (or the preservation) of life." These, in short, address the problem of monoculture or the use of one taxon (cultivar, clone, hybrid, etc.) only. This is a "cat and mouse game," since the pathogen is constantly generating new pathogenic forms, and the fungus has the advantage over the host since the turnover in producing new pathogenic strains is much faster than can be done in the host.

7. Beetle traps using pheromones have been used effectively in some cases, but there are associated problems with it: fungal-contaminated beetles can be lured **into** a stand of healthy elms, rather than **away from** them. Wind direction is also often involved when traps are used.

8. Crown sprays were very effective when such products as DDT (dichlorodiphenyltrichloroethane) and methoxychlor (1,1,1-Trichloro-2,2-bis(4-methoxyphenyl)ethane) were used, but they have been banned because of environmental hazards. In some regions, permethrin-like compounds have been used successfully, but I am not sure if they are still being used, and if so how effective they are. The plant-derived insecticides are more acceptable environmentally as they have short residual activity, as opposed to DDT, methoxychlor, and other "old chemistries."

B. Therapy or the treatment of established disease (infection) has been used successfully in only a very few cases, and so for this reason pathologists always emphasize prevention over cure. To eliminate established infection, detection must be made very early at the earliest documentation, and infusion initiated immediately. This concept is tantamount to curing diseases (microbial infectious or some cancers) in humans and other animals. The major elusive problem is deciding if chemotherapy should or can be administered to the oftentimes extensive vascular infection that is nonvisible to the eye, as bark covers the xylem in which infection occurs. When infection reaches the tree base (shoot/root interface), a type of "metastatsis" occurs, and the fungal propagules are distributed in many directions into many vulnerable tissues. This writer came down with tick-mediated Lyme disease while volunteering in the Shenandoah National Park, and the immediate administration by his physician of doxycycline provided a complete cure.

I successfully cured the large American elm on the northeast lawn at Mt. Vernon (George Washington's home near Alexandria, VA). Detection in June 1979 was followed immediately with treatment using MBC (methyl-2-benzimidazole carbamate) phosphate as large volume infusions coupled with radical surgery. MBC is a highly anti-fungal derivative of benomyl fungicide. I also cured a large elm on the campus of Virginia Tech, one of the first cures attempted that I know of. Successful chemotherapy must be implemented at the very start of infection. In tracing the progress of vascular infection, it is well known that the vascular lesion is often present well beyond the visible foliar symptoms.

My colleague, the late Richard J. Campana, did some studies on the use of radical surgery alone in curing the tree of DED, which involves the immediate removal of symptomatic branches. Success was realized, but again knowing where and how to perform the surgery can be tricky as it is difficult, if not impossible, to determine where the infection exists in the symptomatic branch. There is a commonality of this procedure and in certain forms of human cancer, that is, early detection and immediate activity are keys to success.

Radical surgery and chemotherapy are handmaidens, and remarkable success has been realized when they are used wisely together.

Integrated pest/disease management can fit under either or both of the above categories, since it embraces both preventive and therapeutic activities. All weaponry and procedures should be employed to achieve maximum results in managing DED.

CANOPY DECLINE ASSESSMENTS IN AMERICAN ELM AFTER INOCULATION WITH DIFFERENT DOSES OF *OPHIOSTOMA ULMI* AND *O. NOVO-ULMI*

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Abstract.—Restoration of American elm (*Ulmus americana* L.) in natural and urban landscapes necessitates the development of new selections that not only exhibit Dutch elm disease (DED, caused by the fungal pathogen *Ophiostoma novo-ulmi* and *O. ulmi*) tolerance, but also an increase the genetic variability of tolerant elms. Toward this end, our program tests DED tolerance of large survivor American elms, crosses between DED-tolerant American elms, and crosses between large survivor and DED-tolerant elms. Accurate phenotyping is critical to accurately assess DED-tolerance. This study examined 1) the effect of different DED pathogen doses; 2) American elm responses to two inoculation timings; and 3) the 8-week DED-induced canopy decline response of 29 American elms selections planted at the Delaware, OH, Forestry Sciences Laboratory. Results suggest a significant dose effect in which the treatment group receiving high levels of DED inoculum exhibited significantly more DED-induced foliar symptoms relative to trees receiving low dosage rates. Furthermore, there is considerable variability in the DED-induced canopy decline ratings associated with the timing of the inoculation. Finally, we observed differences in DED-induced canopy decline between selections of large survivor trees collected around the Midwest, indicating that unique tolerance mechanisms may be present in the natural elm population.

Introduction

The American elm (*Ulmus americana* L.) was once widely distributed throughout the eastern United States before the arrival of Dutch elm disease (DED), caused by the fungal pathogens *Ophiostoma ulmi* (Buisman) C. Nannf. and *O. novo-ulmi* Brasier. American elm's tall height coupled with its vase-like shape provides for a uniquely graceful tree that was commonly planted along city streets and boulevards. The crowns of mature elms spanned countless roadways, houses, and recreation areas, where they provided the benefits of cleaner air and cooler temperatures. American elm is one of the few native tree species capable of thriving in the harsh urban environment, where extreme summer temperatures, air pollution, and road salt are common. Before the invasion of DED, elm was an ecologically important tree species in riparian areas and bottomlands, stabilizing riparian slopes against seasonal flooding and enriching soils through the production of rapidly decomposable nutrient-rich leaf litter. Finally, its seeds were an important source of food for song birds and other early migratory birds, as elm seeds matured in the spring before most other seeds are available.

The DED fungal pathogen *O. ulmi* was introduced into the United States in 1930 and its spread has devastated North American species of elm, severely reducing the use of American elm as an urban shade tree. In Illinois in the 1940s the Eurasian race of *O. novo-ulmi* appeared causing a second wave of elm mortality. Research on American elm from the 1970s to the present has focused on the identification of American elm selections that could withstand the

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DED pathogen. Of the more than 100,000 American elm trees tested for resistance to DED, very few selections exhibited adequate levels of DED tolerance. While a few selections are commercially available, most of the elms purchased in the United States are 'Princeton' elms. The widespread use of few DED-tolerant clones presents the risk of another wave of elm mortality due to attacks by other pests/pathogens or mutation of the DED pathogen. Additional DED-tolerant selections representative of the genetic diversity of native American elm populations and suitable for both urban and forested settings are needed to ensure the long-term stability of DED-tolerance among American elm populations. Toward this goal, several research programs have carried out work on the selection and breeding of American elms (Schreiber and Domir 1994; Sherald 1993; Smalley et al. 1993; Smalley and Guries 1993; Townsend 2000; Townsend et al. 2005, 1995), though all have largely ended due to retirements and limited funding.

We are engaged in an ongoing study to identify and generate additional American elm selections that can tolerate DED pathogens. Our approach is twofold: to test DED tolerance of large surviving American elm trees, and to cross these elms with known DED-tolerant elms in order to develop genetically diverse and regionally adapted DED-tolerant American elm populations. This paper describes the results from three complementary experiments: 1) a DED inoculation trial of American elm selections with low, high, and very high doses of a mixture of *O. ulmi* and *O. novo-ulmi*; 2) an experiment investigating differential responses of American elm selections (Kuhar 1 and 2) inoculated in the early summer (June) and late summer (August); and 3) a test of the DED tolerance of 29 American elm selections.

Materials and Methods

To test the response of American elm selections to different DED pathogen dosage rates, six American elm clones from each selection were clonally propagated. Five of these selections (ND104, NR496, NV17, NR521, and NV463) are from DED-tolerant × DED-tolerant crosses and the sixth (SL32) is from a large survivor tree from Michigan (n=137, between 22 and 26 per selection). Elms were planted in two tree orchards at the Delaware, OH, U.S. Forest Service Forest Science Laboratory between 2005 and 2011. Elm trees were inoculated with a 50–50 mixture of *O. ulmi* and *O. novo-ulmi* spores on June 7 and 8, 2016. The inoculum was prepared a week in advance from frozen cultures of *O. ulmi* (strain PG442) and *O. novo-ulmi* (strain H961) as described in Pinchot et al. (in press). Trees in field plots received either a low DED dose of 6×10^5 *O. ulmi* and *O. novo-ulmi* spores, or a high dose of 1.2×10^6. A cordless drill with a 0.47-cm-diameter brad point bit was used to drill a 1.3-cm deep hole 30 cm from the base of trees, and the fungal spores were pipetted into the hole. The canopies of field-grown elms were cleared of any dead branches at the time of inoculation. As such, all trees had baseline measurements of 0 percent canopy decline. Each tree was remeasured 8 weeks post-inoculation. Canopies were rated at 5 percent decline classes (i.e., 0, 5, 10…95, 100 percent) for DED symptoms. Typical DED symptoms consist of foliar yellowing, wilting (flagging), and eventual browning as a branch dies. Because a subset of the trees was split between two tree orchards, we tested differences in the tree canopy decline ratings between the orchards with an analysis of variance (ANOVA) with orchard and selection (n=3) as the main factors. As the canopy decline of three well-replicated selections were similar between two tree orchards (ANOVA; P=0.082) this factor was excluded from all future analyses. Following this, we tested for a DED dosage treatment effect using a mixed model ANOVA with dose (low vs. high) and selection (ND104, NR496, NV17, NR521, NV463 and SL32) as our main factors and a dose*selection interaction. Differences within main factors were analyzed using post-hoc Tukey's honestly significant difference tests (α=0.05).

To test the response of American elms to the timing (and rate) of DED-inoculation, 10 Kuhar (1 and 2, n=5 each) trees were inoculated with a 50–50 mixture of *O. ulmi* and *O. novo-ulmi* spores. Trees were 9 and 12 years old depending on the time of inoculation; d.b.h.: 7.15 ± 0.56 cm [mean ± SE]. The methods outlined above were followed for the elms inoculated June 8, 2016. For the elms inoculated August 13, 2013, a total of 16×10^6 spores were placed into three equidistant holes drilled at a height of 1 m from the base of the tree. In each year, canopy decline was measured as described above at 8 weeks. To analyze differences between the foliar responses of Kuhars inoculated with DED at different times in the summer, we first utilized a t-test to analyze for differential decline between Kuhar 1 and Kuhar 2. No significance was found (P>0.05) and we compiled all data from each time for a two-tailed t-test of foliar decline between August 2013 and June 2016.

Finally, as part of a large-scale DED screening efforts, we clonally propagated large survivor American elm trees (n=29 selections) found in Michigan, Ohio, Illinois, and Indiana, in addition to American elm generated from DED-tolerant selections (cross progeny trees) as described above. These trees (n=497) were planted in replicated blocks and ranged in diameter from 1.2 to 13.9 cm at breast height (diameter at 1.27 m from ground; d.b.h.) and in height from 1.4 to 9.96 m. On June 7, 2016 (as described above) elms we inoculated with the low dose of DED inoculum (6×10^5 spores) to test differential responses to DED exposure. Again, we measured canopy decline after 8 weeks and compared the percentage canopy decline between selections using an ANOVA model with block nested within plot and selection as main effects, and d.b.h. as a covariate. Post-hoc pairwise comparisons of canopy decline between selections were conducted using Bonferroni adjustments (α=0.05).

Results & Discussion

We observed significantly lower rates of DED-induced canopy decline between trees inoculated with a low level of DED (6×10^5 spores, 14.4 percent foliar symptoms) relative to those inoculated with a high level (1.2×10^6 spores, 26.5 percent foliar symptoms) (Figure 1A; ANOVA P<0.001). As expected, we observed differential decline between the selections with SL32 (>45 percent foliar symptoms) and NV463 (25 percent) exhibiting the highest level of DED-induced foliar symptoms (Fig 1B; P<0.001). No significant interaction was observed, suggesting similar responses across all selections to the increased dose (P=0.079). Despite the lack of a significant interaction effect, the selections which exhibited the lowest levels of DED tolerance (NV463 and SL32) performed worse under the high DED inoculation rate relative to the low rate. Interestingly, there was not an enhanced decline in NV17 or NR521 to the increased DED dosage rate, suggesting opportunities for future exploration. Considering ongoing DED inoculation trials, the implications of this dosage effect suggest that researchers should consider rates such as the 1.2×10^6 spores used above to elicit stronger responses in elms. It should be considered however that the optimal dose may vary with DED strain and the ratio of *O. ulmi*: *O. novo-ulmi*.

It has long been suspected that there is a seasonal effect of DED and that exposure during the early summer (in part because of growth, acropetal water and nutrient transmission, and general physiology) may be more harmful to elms than a late summer/fall exposure (Pomerleau 1965, Smalley and Guries 1993). Our results indicate that early June exposure results in significantly enhanced canopy decline relative to late summer/fall exposure, even despite the difference in dosage rates within the study (6×10^5 in spring vs. 16×10^6 in fall, Figure 2). While our replication was low, these results suggest that when undertaking DED tolerance testing, care should be taken to challenge elms during the period when they are most susceptible.

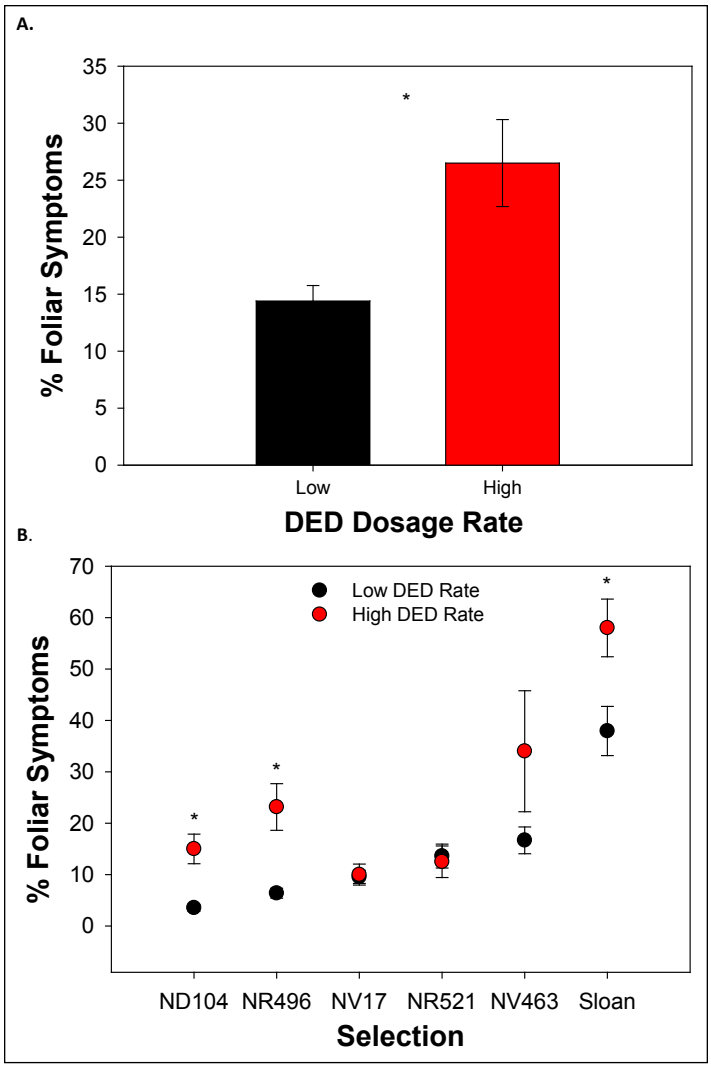

Figure 1.—A. Elm foliar symptoms 8-weeks following inoculation with low (black) and high (red) rates of *O. ulmi* and *O. novo-ulmi*. B. Differential elm foliar symptoms 8-weeks following DED inoculation in six selections exposed to low (black) and high (red) DED inoculation rates. Values represent means ± SE. Asterisk denotes significant difference between the low and high rate ($P<0.05$).

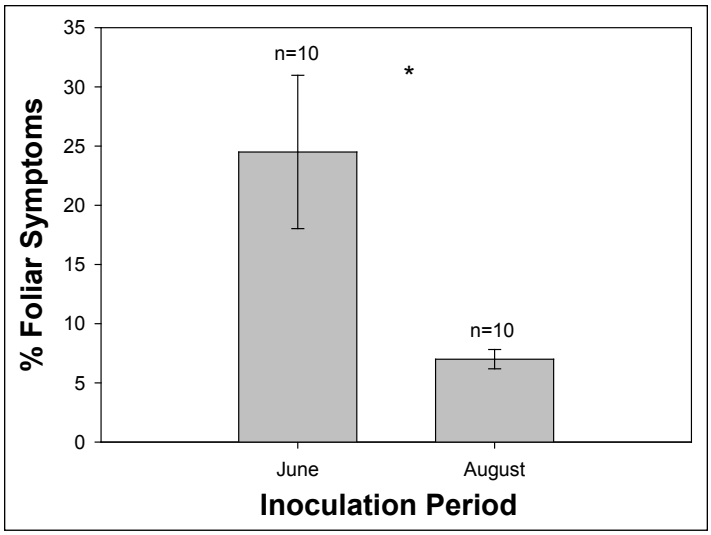

Figure 2.—Eight-week foliar symptoms following DED inoculation of Kuhar (1 & 2). Values represent means ± SE, asterisk denotes significant difference between June and August ($P=0.015$).

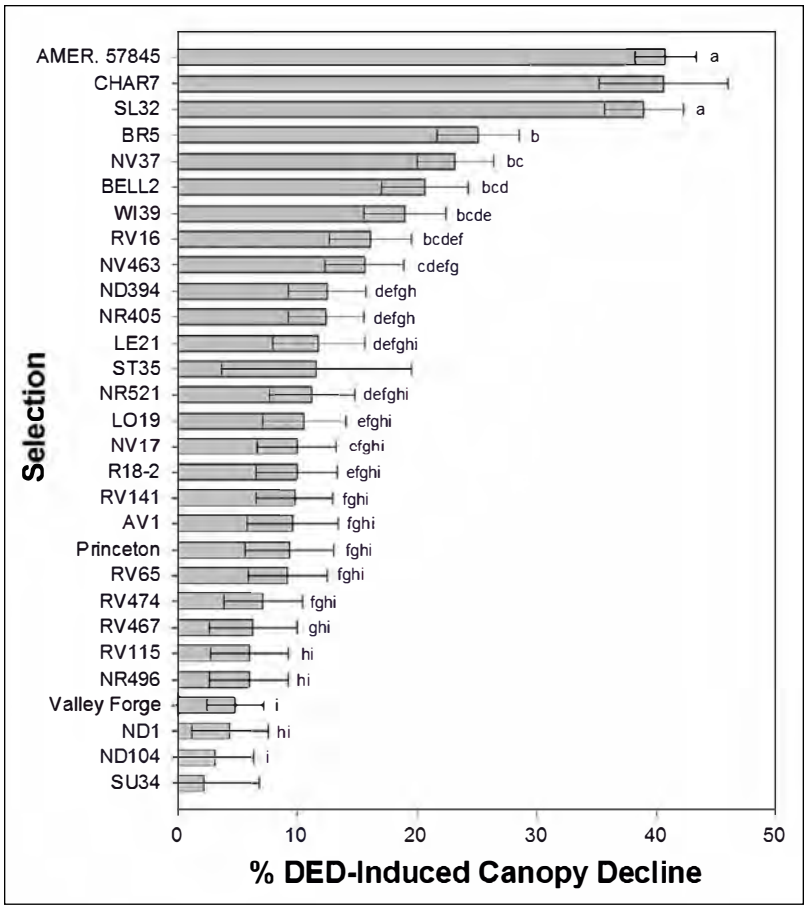

Figure 3.—Eight-week foliar symptoms following DED inoculation of American elm selections. Superscripts denote significant differences between cultivars (P<0.05), cultivars without letters were excluded from the model because of insufficient replication across blocks.

Results of our large-scale tree screening trial indicates considerable variability in canopy decline between the selections 8-weeks post-inoculation (Figure 3; ANOVA, P<0.001). Canopy decline ranged from <5 percent decline (in SU34, ND104, ND1, and 'Valley Forge') to ~40 percent (known susceptible Amer. 57845, SL32 and CHAR7). Furthermore, several selections performed as well as existing commercial cultivars ('Valley Forge' and 'Princeton'). The variability in performance highlights that moderate DED tolerance is exhibited in many selections and that continued breeding may enhance tolerance levels by stacking genes associated with tolerance mechanisms within new selections.

In summary, these results highlight the variability in decline symptoms that can be observed during DED inoculations conducted under differing conditions. To make DED-inoculation data cross comparable between studies, care must be taken to inoculate individuals at a similar time of year and with a consistent amount of inoculum. Findings herein suggest that the high inoculation rate (1.2×10^6) elicits a higher decline rate (relative to the low rate), and thus produces a more stringent tolerance test. More testing should be conducted to compare different strains and investigate a strain x dose interaction. The seasonal effect described herein should be used to guide optimal inoculation times for tolerance trials and suggests that early season inoculations elicit a higher response. Finally, results from the 2016 elm screening indicate considerable variability in the DED tolerance and that several large surviving elms performed as well as the commercially available American elms ('Valley Forge' and 'Princeton').

Acknowledgements

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References

Pinchot, C.C.; Flower, C.E.; Knight, K.S.; Marks, C.; Minocha, R.; Lesser, D.; Woeste, K.; Schaberg, P.G.; Baldwin, B.; Delatte, D.; Fox, T.; Hayes-Plazolles, N.; Held, B.; Lehtoma, K.; Long, S.; Mattix, S.; Sipes, A.; Slavicek, J.S. **Development of new Dutch elm disease-tolerant selections for restoration of the American elm in urban and forested landscapes.** In: Gene Conservation of Trees. In press.

Pomerleau, R., 1965. **The period of susceptibility of *Ulmus americana* to Ceratocystis ulmi under conditions prevailing in Quebec.** Canadian Journal of Botany. 43: 787-792.

Schreiber, L.R.; Domir, S.C. 1994. **Efficacy of criteria to identify aggressiveness in *Ophiostoma ulmi* and resistance in American elm germ plasm.** Plant Disease. 78: 629-632.

Sherald, J.L. 1993. **Demands and opportunities for selecting disease-resistant elms.** In: Sticklen, M.B.; Sherald, J.L., eds. Dutch elm disease research: cellular and molecular approaches. Springer-Verlag, New York, NY: 60-68.

Smalley, E.B.; Guries, R. 1993. **Breeding elms for resistance to Dutch elm disease.** Annual Review of Phytopathology. 31: 325-352.

Smalley, E.B.; Guries, R.P.; Lester, D.T. 1993. **American Liberty elms and beyond: going from the impossible to the difficult.** In: Sticklen, M.B.; Sherald, J.L., eds. Dutch elm disease research: cellular and molecular approaches. Springer-Verlag, New York, NY: 26-45.

Townsend, A.M. 2000. **USDA genetic research on elms.** In: Dunn, C.P., ed. The elms: breeding, conservation, and disease management. Boston, MA: Kluwer Academic Publishers: 271-278.

Townsend, A.M.; Bentz, S.E.; Douglass, L.W. 2005. **Evaluation of 19 American elm clones for tolerance to Dutch elm disease.** Journal of Environmental Horticulture. 23(1): 21-24.

Townsend, A.M.; Bentz, S.E.; Johnson, G.R. 1995. **Variation in response of selected American elm clones to *Ophiostoma ulmi*.** Journal of Environmental Horticulture. 13(3): 126-128.

EFFECTS OF INOCULATION TIMING ON SYMPTOM DEVELOPMENT IN *ULMUS AMERICANA* L.

Garrett L. Beier, Benjamin W. Held, Chad P. Giblin, and Robert A. Blanchette[1]

Abstract.—Field inoculation trials are an important component of screening American elms (*Ulmus americana*) for levels of resistance to Dutch elm disease. A major concern in screening is variability in disease ratings from year to year. Previous studies have demonstrated that timing of inoculation can have a significant impact on disease susceptibility. In this study, trees were inoculated in the main stem using a drill method of inoculation. A recently collected isolate of *Ophiostoma novo-ulmi* with known pathogenicity was used for inoculations. Three different inoculation times were examined: early (May 26), mid (June 23), and late (August 4) season. Trees were assessed for wilt symptoms at 2, 4, 6, and 8 weeks post inoculation using a disease severity scale of 1-6. The trees in the early season inoculation group had the highest mean disease severity ratings at 4, 6, and 8 weeks post inoculation (WPI), while the late season inoculation group had the lowest disease rating at every time point as well as the smallest area under the disease progress curve. Scientists evaluating American elms for resistance to Dutch elm disease should avoid late season inoculations due to reduced disease susceptibility.

Introduction

American elm, *Ulmus americana* L., populations have been decimated by the introduction of *Ophiostoma ulmi* (Buisman) Melin & Nannf. and *O. novo-ulmi* Brasier. Due to the significant losses, there is interest in selecting, developing, and releasing American elm cultivars with higher levels of resistance compared to susceptible genotypes. Before being released to the public, cultivars generally undergo repeated testing to determine their relative resistance to *O. novo-ulmi*. In order to test genotypes for resistance, artificial inoculations are frequently used (Mittempergher and Santini 2004, Smalley and Guries 1993, Solla et al. 2005a).

Previous studies have demonstrated that a number of variables can impact disease development in artificially inoculated elms (Solla and Gil 2002, Solla et al. 2005b, Sutherland et al. 1997, Tchernoff 1965). The variable examined in this study is the timing of inoculation. There have been multiple studies which have examined the impact of timing of inoculation on disease development in American elms (Pomerleau 1965, Smalley 1963, Smalley and Kais 1966, Smalley and Lester 1983, Takai and Kondo 1979). However, the studies were conducted more than 30 years ago, and it would be advantageous to determine if utilizing current isolates would result in differences from previous findings. Brasier (1996) and Plourde and Bernier (2014) examined pathogenicity of *Ophiostoma novo-ulmi* isolates from North America, but the most recent isolate examined in either study was from the mid-1990s.

The goal of this study is to determine if timing of inoculation significantly impacts disease development in artificially inoculated American elm trees using an isolate recently collected from a diseased elm. If differences exist in disease severity based on different inoculation times, which has been evident in previous studies, consideration should be given to utilize inoculation times

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that maximize disease severity in order to effectively determine the levels of resistance within a given genotype. By inoculating during times of greatest susceptibility, there should be greater continuity in results between trials performed in different years and locations.

Materials and Methods

Twenty-four *Ulmus americana* trees were used for this study, 16 trees from a Minnesota seed source and 8 trees from an Ontario, Canada, seed source. Seed from both locations were generated through open pollination. Trees were transplanted in a nursery field at the University of Minnesota, St. Paul campus, on July 6, 2014. During the growing season, the trees were watered as needed and received 4.9 ml of Osmocote® Plus (15-9-12) (Everris NA Inc., Dublin, OH) every 3 months to ensure adequate access to nutrients. At the time of inoculation, trees were 3-4 m tall and approximately 2-4 cm d.b.h.

A Minnesota isolate of *Ophiostoma novo-ulmi*, with known pathogenicity, was used for inoculations. After 7 days of growth on selective media for *Ophiostoma* described in Harrington (1981), three 0.5-cm^2 pieces of colonized media were added to 100 ml of liquid media described in Stennes (1981). Cultures were placed on a shaker at 150 rpm and allowed to grow for 3 days at room temperature. Spore suspension concentrations were determined using a hemocytometer and adjusted to 1×10^6 spores/ml (Buiteveld et al. 2015). This process was repeated for each inoculation.

There were three treatments based on when they were to be inoculated: early, mid, and late season. Each treatment contained eight trees, five randomly selected trees from the Minnesota, seed source and three randomly selected trees from the Ontario seed source. Due to limited plant material, the mid inoculation treatment had six trees from the Minnesota seed source and only two from the Ontario seed source. For each treatment, six trees were inoculated with a spore suspension and one tree from each of the two seed sources were inoculated with sterile water to serve as controls. The early season inoculation group was inoculated on May 26, 2016 (40 days after budbreak), the midseason inoculation group on June 23, 2016 (68 days after budbreak), and the late season inoculation group on August 4, 2016 (110 days after budbreak). Inoculations were made using a drill method modified from a study by Townsend et al. (2005). Briefly, trees were inoculated by drilling a 4 mm deep hole with a 2.4 mm diameter drill bit 0.5 m above the ground on the main stem (Fig. 1). Twenty-five µm of the spore suspension containing 1×10^6 spores/ml were injected into the hole using a micropipette and sealed with Parafilm M® (Bemis Co., Neenah, WI) to avoid desiccation.

Figure 1.—A drill was used to make a wound 0.5 m above the ground for inoculations. Photo by Benjamin Held, used with permission.

Figure 2.—A representative tree in the early inoculated group at 3 weeks post inoculation displaying permanent wilt in a majority of the crown. Photo by Garrett Beier, used with permission.

Disease symptoms were assessed every 2 weeks following inoculation. Disease ratings were based on the percentage of the crown exhibiting permanent wilt (Fig. 2). Ratings were made on a 1–6 ordinal scale: 1=0 percent wilt; 2=1 to 25 percent wilt; 3=26 to 50 percent wilt; 4=51 to 75 percent wilt; 5=75 to 99 percent wilt; and 6=100 percent wilt. Area under the disease progress curve (AUDPC) was measured using the mean disease severity ratings at 2, 4, 6, and 8 weeks post inoculation for each treatment. Calculating AUDPC is a useful method to determine disease intensity over time (Campbell and Madden 1990, Shaner and Finney 1977).

Analysis was performed using the statistical package R version 3.2.2 (R Development Core Team, Vienna, Austria). Data on disease severity was measured using an ordinal scale and often lacked normal distribution based on Shapiro-Wilk Normality Test results. To account for repeated measures, the F1 LD F1 macro from the nparLD package (Noguchi et al. 2012) was used to calculate an analysis of variance-type statistic (ATS), which is a nonparametric method to test treatment, time, and treatment x time interaction effects. The use of ATS for nonparametric analysis of repeated measures is described in Shah and Madden (2004). Since the treatment effect was found to be significant, treatments were compared at each time point using the Kruskal-Wallis test followed by Dunn's multiple comparisons test with a Benjamini and Hochberg (1995) *p*-value adjustment. Area under the disease progress curve data was analyzed using ANOVA followed by the Fisher's LSD test with a Benjamini and Hochberg (1995) *p*-value adjustment.

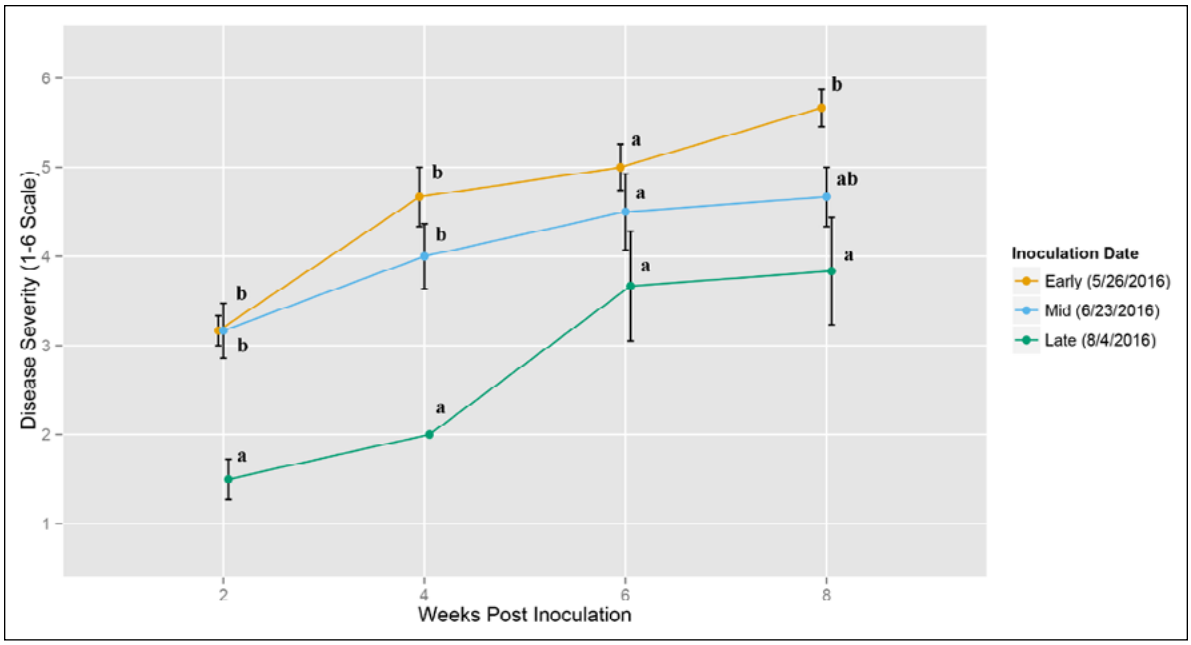

Figure 3.—Effect of timing of inoculation in *Ulmus americana* on biweekly disease severity ratings. Points represent the mean of six trees and bars represent the standard error of the mean. Groups containing the same letter within a column are not significantly different according to Dunn's multiple comparison test with a Benjamini and Hochberg p-value adjustment (α=0.05).

Results

There was a significant effect of timing of inoculation on disease severity rating (p<0.001). The early season inoculation group had the highest average disease rating at 4, 6, and 8 weeks post inoculation (WPI), while the late season inoculation group had the lowest average disease rating for every time point. Although trees in the early season inoculation group had a higher average disease rating compared with the midseason inoculation group at 4, 6, and 8 WPI, differences in the populations were not found to be statistically significant (p>0.05). At 2 and 4 WPI, there was a statistically significant difference between late season inoculations and the early and midseason inoculations for disease severity. At 8 WPI, the late season inoculation group had an average wilt rating of 3.8, while the early inoculation group averaged 5.7, and the difference between the groups was found to be statistically significant (p<0.05) (Fig. 3). The number of trees at 100 percent permanent wilt at 8 WPI varied depending on the timing of inoculation. For the early season group 4 of 6 trees had 100 percent wilt, for the midseason group only 1 of 6 trees had 100 percent wilt, and none of the late season inoculated trees had 100 percent wilt.

Disease progression was also affected by timing of inoculation. The late season inoculation group displayed a slower progression of disease compared with the early and midseason inoculations. Area under the disease progress curve at 8 WPI for the late season inoculation group was 10.7 and significantly less than that of the early season and midseason inoculations, 22.2 and 18.8 respectively (Table 1).

Table 1.—Effect of timing of inoculation in *Ulmus americana* on area under the disease progress curve (AUDPC) at 8 weeks post inoculation

Inoculation group	Inoculation date	Mean[a] ± SE
Early	5/26/16	22.2 ± 1.4 b
Mid	6/23/16	18.8 ± 2.0 b
Late	8/4/16	10.7 ± 1.9 a

[a] Means containing the same letter are not significantly different according Fisher's LSD test (α=0.05).

Discussion

A major concern for researchers testing elm genotypes to evaluate resistance to DED has been a lack of consistency between years and locations carried out in different trials. Findings from this study show that American elms display different susceptibility to infection with *Ophiostoma novo-ulmi* depending on the timing of inoculation. These results confirm studies completed by others (Pomerleau 1965, Smalley 1963, Smalley and Kais 1966, Smalley and Lester 1983, Takai and Kondo 1979). To maintain consistency and effectiveness in screening, it is advisable to inoculate trees when they are at their greatest susceptibility to infection. Alternatively, using the same susceptible and resistant controls across experiments could help investigators assess resistance of different genotypes inoculated at different times by using the controls as baselines. Ideally, controls used in resistance studies would be clones of a genotype in order to reduce potential variability in disease susceptibility due to genetics. One limitation to this study is seedlings were used instead of clones, which may have been an additional source of variation in disease susceptibility.

Investigators have used terms such as the greatest and highest level of susceptibility when referring to inoculation timing (Pomerleau 1965, Smalley and Kais 1966, Takai and Kondo 1979). The use of these terms is problematic, as they do not have a universal definition. Should greatest susceptibility be based on the inoculation time when the highest percentage of trees show visible wilt symptoms when later rated or when the trees display the highest mean wilt symptoms when later rated? If mean wilt symptoms are to be used, how long after inoculation should trees be rated for wilt symptoms? For the purpose of this study, the time of greatest susceptibility was considered the inoculation time that resulted in highest mean percent wilt 8 WPI. Additional studies with more inoculation time periods, such as every week, could be used to more accurately determine the time of greatest susceptibility.

A common finding amongst scientists who have performed studies to examine the effect of timing of inoculation is that results vary depending on year (Pomerleau 1965, Smalley and Kais 1966, Tchernoff 1965). Although the recommended use of calendar dates or days after budbreak allows for simplicity in inoculation timing, variation in weather from different years and locations, limits its reliability. A method to help reduce the variability caused by weather conditions would be to use growing degree days. Takai and Kondo (1979) conducted a study examining the effects of timing of inoculation on disease susceptibility. After examining disease severity and mortality they calculated the growing degree days which corresponded to the inoculation dates for the beginning and end of greatest susceptibility. A critical component of calculating growing degree days is base temperature. Takai and Kondo (1979) arbitrarily selected 5.6 °C as their base temperature. Mathematical equations are available to determine the appropriate base temperature for growing degree days (Yang et al. 1995), however, before a base temperature can be determined, the inoculation time of greatest susceptibility must be defined. For future studies investigating the effects of timing of inoculation on symptom development,

we suggest including the location of the trial, the date of budbreak, and the 8 WPI wilt rating so results from this study can be combined with that of others to more accurately determine the number of growing degree days to the time of greatest susceptibility. Factors other than timing have also been shown to affect periods of greatest susceptibility. Smalley and Kais (1966) found that plant size as well as inoculation method, branch versus trunk inoculations, impacted the duration and timing of susceptibility. Therefore, caution should be used when comparing experiments examining resistance when different methods were used.

Acknowledgments

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Literature Cited

Benjamini, Y.; Hochberg, Y. 1995. **Controlling the false discovery rate: a practical and powerful approach to multiple testing.** Journal of the Royal Statistical Society, Series B. 57: 289-300.

Brasier, C.M. 1996. **Low genetic diversity of the *Ophiostoma novo-ulmi* population in North America.** Mycologia. 88: 951-964.

Buiteveld, J.; Van Der Werf, B.; Hiemstra, J.A. 2015. **Comparison of commercial elm cultivars and promising unreleased Dutch clones for resistance to *Ophiostoma novo-ulmi*.** iForest. 8: 158-164.

Campbell, C.L.; Madden, L.V. 1990. **Introduction to plant disease epidemiology.** New York, NY: Wiley-Interscience. 532 p.

Harrington, T.C. 1981. **Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*.** Mycologia. 73: 1123-1129.

Mittempergher, L.; Santini, A. 2004. **The history of elm breeding.** Investigación Agraria. Sistemas y Recursos Forestales. 13: 161-177.

Noguchi, K.; Gel, Y.R.; Brunner, E.; Konietschke, F. 2012. **nparLD: An R software package for the nonparametric analysis of longitudinal data in factorial experiments.** Journal of Statistical Software. 50: 1-23.

Plourde, K.V.; Bernier, L. 2014. **A rapid virulence assay for the Dutch elm disease fungus *Ophiostoma novo-ulmi* by inoculation of apple (*Malus* × *domestica* 'Golden Delicious') fruits.** Plant Pathology. 63: 1078-1085.

Pomerleau, R. 1965. **The period of susceptibility of *Ulmus americana* to *Ceratocystis ulmi* under conditions prevailing in Quebec.** Canadian Journal of Botany. 43: 787-792.

Shah, D.A.; Madden, L.V. 2004. **Nonparametric analysis of ordinal data in designed factorial experiments.** Phytopathology. 94: 33-43.

Shaner, G.; Finney, R.E. 1977. **The effect of nitrogen fertilizer on the expression of slow-mildewing resistance in Knox wheat.** Phytopathology. 67: 1051-1056.

Smalley, E.B. 1963. **Seasonal fluctuations in susceptibility of young elm seedlings to Dutch elm disease.** Phytopathology. 53: 846-853.

Smalley, E.B.; Guries, R.P. 1993. **Breeding elms for resistance to Dutch elm disease.** Annual Review Phytopathology. 31: 325-352.

Smalley, E.B.; Kais, A.G. 1966. **Seasonal variations in the resistance of various elm species to Dutch elm disease.** In: Gerhold, H.D.; McDermott, R.E.; Schreiner, E.J.; Winieski, J.A., eds. Breeding pest-resistant trees. London, UK: Pergamon Press Ltd.: 279-292.

Smalley, E.B.; Lester, D.T. 1983. **'Regal' elm.** HortScience. 18: 960-961.

Solla, A.; Bohnens, J.; Collin, E.; Diamandis, S.; Franke, A.; Gil, L.; Burton, M.; Santini, A.; Mittempergher, L.; Pinon, J.; Broeck, A.V. 2005a. **Screening European elms for resistance to *Ophiostoma novo-ulmi.*** Forest Science. 51: 134-141.

Solla, A.; Gil, L. 2002. **Influence of water stress on Dutch elm disease symptoms in *Ulmus minor.*** Canadian Journal of Botany. 80: 810-817.

Solla, A.; Martin, J.A.; Ouellette, G.B.; Gil, L. 2005b. **Influence of plant age on symptom development in *Ulmus minor* following inoculation by *Ophiostoma novo-ulmi.*** Plant Disease. 89: 1035-1040.

Stennes, M.A. 1981. **Thiabendazole hypophosphite and carbendazim phosphate as systemic fungicides for practical Dutch elm disease control.** St. Paul, MN: University of Minnesota. M.S. thesis.

Sutherland, M.L.; Pearson, S.; Brasier, C.M. 1997. **The influence of temperature and light on defoliation levels of elm by Dutch elm disease.** Phytopathology. 87: 576-581.

Takai, S.; Kondo, E.S. 1979. **Seasonal development of Dutch elm disease on white elms in central Ontario, Canada. I. Following wound inoculation.** Canadian Journal of Botany. 57: 341-352.

Tchernoff, V. 1965. **Methods for screening and for the rapid selection of elms for resistance to Dutch elm disease.** Acta Botanica Neerlandica. 14: 409-452.

Townsend, A.M.; Bentz, S.E.; Douglass, L.W. 2005. **Evaluation of 19 American elm clones for tolerance to Dutch elm disease.** Journal of Environmental Horticulture. 23: 21-24.

Yang, S.; Logan, J.; Coffey, D.L. 1995. **Mathematical formulae for calculating the base temperature for growing degree days.** Agricultural and Forest Meteorology. 74: 61-74.

CHALLENGE INOCULATIONS TO TEST FOR DUTCH ELM DISEASE TOLERANCE: A SUMMARY OF METHODS USED BY VARIOUS RESEARCHERS

Linda M. Haugen, Garrett L. Beier, Susan E. Bentz, Raymond P. Guries, and James M. Slavicek[1]

Abstract.—A variety of methods have been used by different research groups to "challenge" inoculate American elms (*Ulmus americana*) with the purpose of determining whether some clones may be resistant to the Dutch elm disease fungus. The methods used by seven research groups are described, along with observations on complications and benefits associated with each. The response of test trees to challenge is affected by many factors, including the age of parent material, size/maturity of test material, vigor of the plant being inoculated, portion of the plant inoculated, season/time of year, source of inoculum, amount of inoculum, and method of delivery. The testing goal must be kept in mind when choosing methods, and the details of what methods were used must be described when reporting results. Inclusion of susceptible and resistant controls is critically important, as it allows calibration of response between different studies.

Introduction

Over the decades, many researchers have used a variety of methods to challenge elm plant material with the Dutch elm disease (DED) pathogens, *Ophiostoma ulmi* (Buisman) and *O. novo-ulmi* (Brasier). The methods have evolved over time, with variations in the methodology even within a particular working group. During the elm workshop, Raymond P. Guries, Garrett L. Beier, Susan E. Bentz, and James M. Slavicek participated in a panel discussion to share information about their standard methods. Alden "Denny" Townsend provided comments in advance. The summary presented here is a combination of information presented by the panel and also captured from related literature. This summary contains an overview of the methodologies used by several working groups, along with some of the complications and benefits associated with each. A discussion section highlights common themes and factors to consider when choosing a challenge protocol.

Note that the terms "resistance" and "tolerance" are both used in this summary, based on that term the particular work group uses. In a human medical sense, the general public tends to interpret "resistance" as meaning that the trees cannot become infected by the pathogen. The definition in plant pathology, however, is the ability to exclude or overcome, completely or to some degree, the effect of a pathogen (Agrios 2005). Townsend (2000) prefers the term "tolerance" because it implies that the pathogen is able to infect the tree but the result in no long-term deleterious effects.

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Overview of Methods

Wisconsin Methods

Beginning in the 1960s and continuing through the 1990s, researchers at the University of Wisconsin were highly active in testing American and other elms for resistance to DED. They used field trials and then also developed a greenhouse screening method (Smalley and Guries 1993).

The field trial approach involved producing rooted cuttings (ramets) of the trees to be tested and then outplanting them to field plots for 3 to 6 years prior to testing. The test trees were challenged by using a hypodermic needle to inject a suspension of 10^6 spore/ml into 0.1-mm drill holes in small branches in the crown. The choice to use small holes on small branches was intended to mimic the type of wound to which bark beetles would introduce the pathogen. Trees were evaluated after 2 to 3 months and again after 1 year post-inoculation. One observation from the years of field testing was that the period of susceptibility was quite variable and was affected by soil moisture, air temperature, age of the plant material, and other factors. Resistance appeared to increase with age while the length of the susceptible period decreased (Smalley and Guries 1993). Field testing required a large area of land and a long period of time; results were highly variable.

Wisconsin also developed a greenhouse methodology to reduce the costs of land and labor, speed up the screening process, and standardize conditions (Green et al. 1984). Root cuttings produce multiple shoots that can be harvested as softwood cuttings after 2 weeks, then rooted and planted into a controlled climate greenhouse. These cuttings generally produce large, vigorous, uniform plants that can be challenge inoculated after 2 to 3 months in the greenhouse. The inoculation method used was a drip hole drilled into the stem, which was then filled to runoff via hypodermic needle with a calibrated dosage (10^6 spores/ml) of mixed spores of *Ophiostoma ulmi* and *O. novo-ulmi.* After 1 to 2 months, the stems of the most susceptible clones exhibited complete mortality, but crown damage was not primarily used for evaluation. Instead, the stems of inoculated shoots were peeled and the extent of discoloration, as measured by height and width of the lesion in the xylem, was recorded as a measure of susceptibility. This approach compressed the testing period to less than 1 year. It was a very aggressive challenge inoculation, but it did allow differentiation in response. Researchers found that rankings of response by clones, families, and accessions were generally similar between greenhouse and field testing approaches. Field trials were still conducted but with fewer clones as the most susceptible were generally not included.

National Arboretum Methods

The methodology of researchers at the Agricultural Research Service (ARS) National Arboretum during the 1990s was field trials with multiple ramets of the test cultivars. Townsend noted[2] that ARS researchers tried the University of Wisconsin methods of inoculation (small wounds in upper crown), but were only able to evoke minor symptoms, so instead they used a main stem wound. This method allowed sufficient foliar symptoms and branch dieback to differentiate the clones. In some studies, material was greenhouse grown for a year then outplanted to the field site for 2 or 3 years prior to inoculation (Townsend et al. 1995). Inoculations were made into a 2.4-mm hole in the lower one-third of the main trunk. The spore suspension consisted of 3×10^6 spores/ml of a mixture of *O. ulmi* and *O. novo-ulmi*. Crown symptoms were recorded 4 weeks after inoculation and dieback was recorded after 1 year. Inoculation earlier in the season resulting in greater crown symptoms. In another study, test cultivars were outplanted to the field site 9 years prior to inoculation, and evaluations were conducted 4 weeks, 1 year, and 2 years after inoculation (Townsend et al. 2005). In some

studies conducted in Ohio, ARS scientists found that trees younger than 3 years had "juvenile resistance".[2]

Forest Service Methods

The inoculation methods currently being used by the U.S. Forest Service Northern Research Station in Delaware, Ohio, are a continuation of methods similar to those previously used by ARS. Ramets of the test material are outplanted to field plots and grown for 3 to 7 years prior to challenge inoculation. The point of inoculation is a hole drilled at an angle downward into the main stem of the tree, approximately 1 foot above soil level. The hole accommodates all of the calibrated spore suspension, so all trees received equal dosage. Small branches are pruned from the main stem for several feet above the point of inoculation so that the spores are not translocated into side branches.

As an example of the specific methodology used in one recent experiment, American elm trees were inoculated with a 50/50 mixture of *O. ulmi* and *O. novo-ulmi* spores on June 7 and 8, 2016. The inoculum was prepared a week in advance as follows: frozen cultures of *O. ulmi* (strain PG442) and *O. novo-ulmi* (strain H961) were thawed and spread on separate potato dextrose agar plates, 50 µl/plate, and nine plates/isolate. The plates were kept dark and at room temperature. Fungal spores were harvested after 11 days of growth by addition of sterile deionized water to the plate surface. The surface was scraped gently with a bent glass rod and the spores of each isolate were removed to a separate sterile 50-ml conical tube. Fungal spore concentrations for each isolate were determined using a hemocytometer. The final 50/50 concentration of spores was adjusted to a volume appropriate for the inoculation of trees. Trees in field plots received either 6×10^5 or 1.2×10^6 spores; potted elms were inoculated with a total of 2.8×10^4 spores. A 0.5 cm diameter brad point bit was used to drill a 1.3 cm deep hole 30 cm from the base of trees located in field plots, and the fungal spores were pipetted into the hole. A 0.2-cm-diameter bit was used to drill a 0.6 cm deep hole 15 cm from the base of potted trees and the fungal spores were pipetted into the hole (Pinchot et al., in press).

Minnesota Methods

University of Minnesota researchers have been focused on screening "survivor elms" for resistance. To generate ramets, dormant shoots are collected and grafted onto established seedling rootstocks to obtain vigorous scionwood. Softwood cuttings are then collected and placed in a peat/perlite mix to promote rooting. After softwood cuttings have developed sufficient roots, they are transplanted to a larger container and subsequently planted in the field for field trials. For most studies, inoculations take place after the test trees have reached a minimum diameter at breast height (d.b.h.) of 2.5 cm. Inoculations are made by using a 2.38-mm drill bit to make a downward slanted hole in the stem, approximately 4 mm deep. Depending on the size of the tree, the hole is filled with 25 to 60 µl of an *Ophiostoma novo-ulmi* spore suspension at 1×10^6 spores/ml. Inoculations are timed to occur at about 40 days following budbreak (Tchernoff 1965), which is usually late May or early June. Based on field trials in 2015 and 2016, it was found that trees were highly susceptible when inoculated approximately 270 growing degree days (base 50) after budbreak. Growing degree days are calculated by averaging the daily maximum temperature and minimum temperature and subtracting the base temperature. If the average of the maximum and minimum temperature is not greater than the base temperature, there are no growing degree days accumulated on that day. Trees are evaluated

[2] Personal communication from Alden Townsend, retired, USDA Agricultural Research Service, U.S. National Arboretum, 3501 New York Ave. NE, Washington, DC 20002.

biweekly for 10 to 14 weeks for percentage of the crown exhibiting permanent wilt following inoculation. Additionally, evaluations are made 1 year post inoculation. Researchers have observed that variation in the timing of inoculations can result in differing levels of susceptibility, with late May inoculations resulting in more severe symptoms.

Greenhouse trials have also been conducted to screen genotypes for resistance. Currently, trees are being screened in the greenhouse and then later in the field to determine if correlations exist between greenhouse screening results and field screening results. Plant material utilized in greenhouse screening experiments are considerably smaller than those in field trials. Generally, plants are inoculated 1 year after being grafted or rooted. Inoculations are made in the same method as described above, except for the size of the inoculation hole and the amount of inoculum injected. Due to the small size of the plant material, 15 to 25 µl of spore suspension at 1×10^6 spores/ml are injected into a 1.59 mm diameter hole approximately 3 to 4 mm deep.

Other Methods Not Addressed During Panel

The methodology used by the National Park Service in Washington, D.C., in the 1960s and 1970s was twig inoculation. Wester (1972) described propagation by grafting buds from select large elm trees onto 2-year-old elm seedlings, then growing the budded plant material for 4 years prior to challenge inoculation. Strong shoots that were 2 years old were selected for inoculation, and inoculations took place about 15 cm below the current growth, during the month of June. Small holes, 1 to 2 mm diameter, were drilled approximately 1 mm deep into the tissue, then flooded with a heavy suspension of *O. ulmi* spores and sealed. Trees were evaluated for wilt symptoms for two seasons after inoculation.

European methods (Tchernoff 1965) involve active sucking of spore suspension into cut xylem elements. For older trees, a utility knife is used to slash the stem, and then while the knife is still in the wound, at least 4 drops of a spore suspension are placed on the wound so that they can be "sucked" into the xylem by vascular tension. On younger trees, a small surgical chisel (with a 2 mm wide point) is used to create a wound into which at least one drop of spores is allowed to be "sucked" in.

Takai and Kondo (1979) compared four inoculation methods, including introduction of spores into drill and 6.5-mm chisel wounds in the lower stem and introduction of spores to a scalpel slit on an upper branch, and pressure injection of spores into a drill hole on an upper branch. The two basal stem methods resulted in more severe and rapid disease development. The two upper branch methods were more similar to the overland transmission by bark beetles. Takai et al. (1979) also infected young elm stems by caging naturally infested native elm bark beetles on the stem, resulting in an inoculation method more representative of natural conditions.

Discussion

Many factors influence the response of test trees to challenge: age of parent material, size/maturity of test material, vigor of the plant being inoculated, portion of the plant inoculated, season/time of year, source of inoculum, amount of inoculum and method of delivery (Takai and Kondo 1979, Tchernoff 1965, and all of the authors involved in writing this summary). There are different methods, but there is no right method. It is important to specify details on what methods were used in testing, including the source of inoculum and timing of testing. Inclusion of susceptible and resistant controls is critically important, as it allows calibration of response between different studies.

The goal of the testing must be kept in mind. More severe testing may eliminate some sources of resistance or tolerance, such as unattractiveness to beetles, which under natural conditions could result in trees being less likely to become infected. Some of these more discrete sources of resistance may be valuable to persistence of American elms in natural forests, even if they are not strong enough to "guarantee" an elm is DED resistant for an urban planting. When publishing results, it is important to interpret the implications of testing methods so that the public does not infer some degree of resistance or tolerance as "immunity."

Age of the plant material at time of testing has often been a topic of discussion. Solla et al. (2005) demonstrated that vascular tissue of *Ulmus minor* under 4 years old was structurally different from older plants, and, correspondingly, DED symptom expression was greater in the older plants. They cite older research papers (Caroselli and Feldman 1951, Neely 1968) that report a similar response in *Ulmus americana*. This supports the popular idea of "juvenile resistance" to DED. However, Wisconsin research (Smalley and Guries 1993) did not necessarily confirm this concept; they were able to establish a correlation between response of juvenile and mature tissue and then used this relationship to enable screening within a shorter timeframe. Minnesota researchers have also observed that young material is highly susceptible. The testing of younger tissue as a means of predicting durable resistance could use further investigation.

Field trials present additional complications. In some locations, herbivory by deer, rabbits, and voles make it difficult to obtain consistent, healthy plant material. Root grafts between adjacent trees can confound inoculation trials. Local populations of naturally occurring DED and elm yellows phytoplasma can also affect studies and testing. Disease development in individual plants is affected by the vigor of the plant, thus as weather, fertilizer, soil moisture content, and other conditions affect plant health, they also affect testing results.

Challenge inoculations do tell us that some American elm trees have a superior ability to survive infection by the DED fungus. We also must consider that challenge inoculation does not give us complete information about the ability of elms to survive long-term on the landscape.

Literature Cited

Agrios, G.N. 2005. **Plant pathology, 5th ed.** London, UK: Elsevier Academic Press. 922 p.

Caroselli, N.E.; Feldman, A.W. 1951. **Dutch elm disease in young elm seedlings.** Phytopathology. 41: 46-51.

Green, C.E.; Guries, R.P.; Smalley, E.B. 1984. **Early screening of elms for resistance to *Ceratocystis ulmi.*** Plant Disease. 69: 60-63.

Neely, D. 1968. **Twig inoculations on American elm with *Ceratocystis ulmi.*** Phytopathology. 58: 1566-1570.

Pinchot, C.C.; Flower, C.E.; Knight, K.S.; Marks, C.; Minocha, R. [et al.]. In press. **Development of new Dutch elm disease-tolerant selections for restoration of the American elm in urban and forested landscapes.** In: Sniezko, R.A.; Man, G.; Hipkins, V. [et al.], tech. cords. Gene conservation of tree species–banking on the future. Proceedings of a workshop. Gen. Tech. Rep. PNW-GTR. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station.

Smalley, E.B.; Guries, R.P. 1993. **Breeding elms for resistance to Dutch elm disease.** Annual Review of Phytopathology. 31: 325-352.

Solla, A.; Martín, J.A.; Ouellette, G.B.; Gil, L. 2005. **Influence of plant age on symptom development in *Ulmus minor* following inoculation by *Ophiostoma novo-ulmi*.** Plant Disease. 89: 1035-1040.

Takai, S.; Kondo, E.S. 1979. **Seasonal development of Dutch elm disease on white elms in central Ontario, Canada. I. Following wound inoculation.** Canadian Journal of Botany. 57: 341-352.

Takai, S.; Kondo, E.S.; Thomas, J.B. 1979. **Seasonal development of Dutch elm disease on white elms in central Ontario, Canada. II. Following feeding by the North American native elm bark beetle.** Canadian Journal of Botany. 57: 353-359.

Tchernoff, V. 1965. **Methods for screening and for rapid selection of elms for resistance to Dutch elm disease.** Acta Botanica Neerlandica. 14: 409-452.

Townsend, A.M. 2000. **USDA genetic research on elms.** In: Dunn, C.P., ed. The elms: breeding, conservation and disease management. Boston, MA: Kluwer Academic Publishers: 271-278.

Townsend, A.M.; Bentz, S.E.; Douglass , L.W. 2005. **Evaluation of 19 American elm clones for tolerance to Dutch elm disease.** Journal of Environmental Horticulture. 23(1): 21-24.

Townsend, A.M.; Bentz, S.E.; Johnson, G.R. 1995. **Variation in response of selected American elm clones to *Ophiostoma ulmi*.** Journal of Environmental Horticulture. 13(3): 126-128.

Wester, H.V. 1972. **Investigation of American elm clons [sic] based on physiological vigor for crown resistance to Dutch elm disease.** Plant Disease Reporter. 56(8): 725-727.

THE GLENWOOD ESTATE: OUR 32-YEAR EXPERIENCE USING ARBOTECT® 20-S TO CONTROL DUTCH ELM DISEASE

William L. MacDonald, Mark L. Double, Cameron M. Stauder, and Kemp Winfree[1]

Abstract.—We report a case study that demonstrates the successful use of the fungicide Arbotect® 20-S to protect American elms (*Ulmus americana*) from Dutch elm disease at a historic site in Charleston, WV. Standard injection protocols were used every 3 to 4 years to deliver the chemical into the root flares. Twelve of the original 16 trees remain 34 years after the initial treatment.

Introduction

Injection of trees with chemicals has been part of the tree care industry for several decades. A component of that technology has been control of vascular wilt diseases (especially Dutch elm disease [DED] and oak wilt) by injection of fungicides (Haugen and Stennes 1999). Over the decades, numerous chemicals have been tested for their efficacy in combating the pathogens that incite these diseases. It wasn't until systemic fungicides, particularly Arbotect 20-S® (thiabendazole; Syngenta Crop Protection LLC, Greensboro, NC) and Alamo® (propiconazole; Syngenta Crop Protection LLC, Greensboro, NC) were developed that acceptable levels of plant vascular disease control was achieved. This report is a case study that demonstrates the usefulness of chemotherapy to preserve high value individual elms.

Our initial efforts to use chemotherapy to control DED occurred in an effort to save several key American elms (*Ulmus americana*) on the West Virginia University (WVU) campus in Morgantown, WV. Initially, we attempted to use a form of the DuPont chemical Benlate® (benomyl) that we solubilized. At the time, this chemical was a widely used systemic fungicide, particularly for agricultural crops. Without much success, we turned to Arbotect 20-S when it became available and early tests by other researchers confirmed its promise as a control for DED. Colleagues at the West Virginia Department of Agriculture (WVDA) were aware of our DED control efforts at WVU. When WVDA was approached about the DED issue at the historic Glenwood Estate in Charleston, WV, they recommended to the board of the West Virginia College of Graduate Studies Foundation (then the controlling body for Glenwood), that we be contacted relative to DED problems. Thus started our three-plus decade of involvement with the Glenwood Estate elms.

The main home on the estate is a majestic Greek revival style mansion that was constructed in 1852 (Fig. 1) (Calwell 2014). The mansion was built on a 148 ha land parcel near the Elk River. The mansion was home to numerous families who figured prominently in the early history of the city of Charleston, WV. Their surnames, including Laidley, Summers, and Quarrier, are encountered today on numerous buildings and streets throughout the city. The original parcel of land functioned as a farm but over the years was subdivided and sold by owners during economic downturns. The mansion has undergone limited restorations over the years but essentially remains much as it did a century and a half ago, including the furnishings. Currently,

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Figure 1.—An early spring photograph of the Glenwood Estate in Charleston, WV. Photo by Brian Marr, used with permission.

the house sits on about 0.8 ha of land and is surrounded by residential neighborhoods. The estate was given to West Virginia College of Graduate Studies Foundation by Lucy Quarrier and Elizabeth Quarrier Hedrick with the intent that it be used for educational purposes. It is listed on the National Register of Historic Places and is managed by the Historic Glenwood Foundation.

There is little evidence or paper records that indicate when the larger trees that exist on the site today were planted; there are records documenting the planting of some of the shrubbery in recent decades. The most common species on the property is American elm (Fig. 2). The elms share the landscape with a few oaks and maples, trees that represent forest species typical of the Kanawha County (West Virginia) region. We presume most of the trees arose as volunteers. Certainly elm figures prominently because of the proximity of the site to the Elk River, an ideal elm ecosystem.

There has been a history of DED and elm yellows in the Charleston area since these diseases were first reported in the late 1930s. Dutch elm disease is frequently observed in Charleston as American elm continues to repopulate the area naturally and many trees succumb each year. Fortunately, elm yellows is rarely observed.

Figure 2.—Several American elms present on the grounds of the Glenwood Estate in Charleston, WV. Photos by Brian Marr, used with permission.

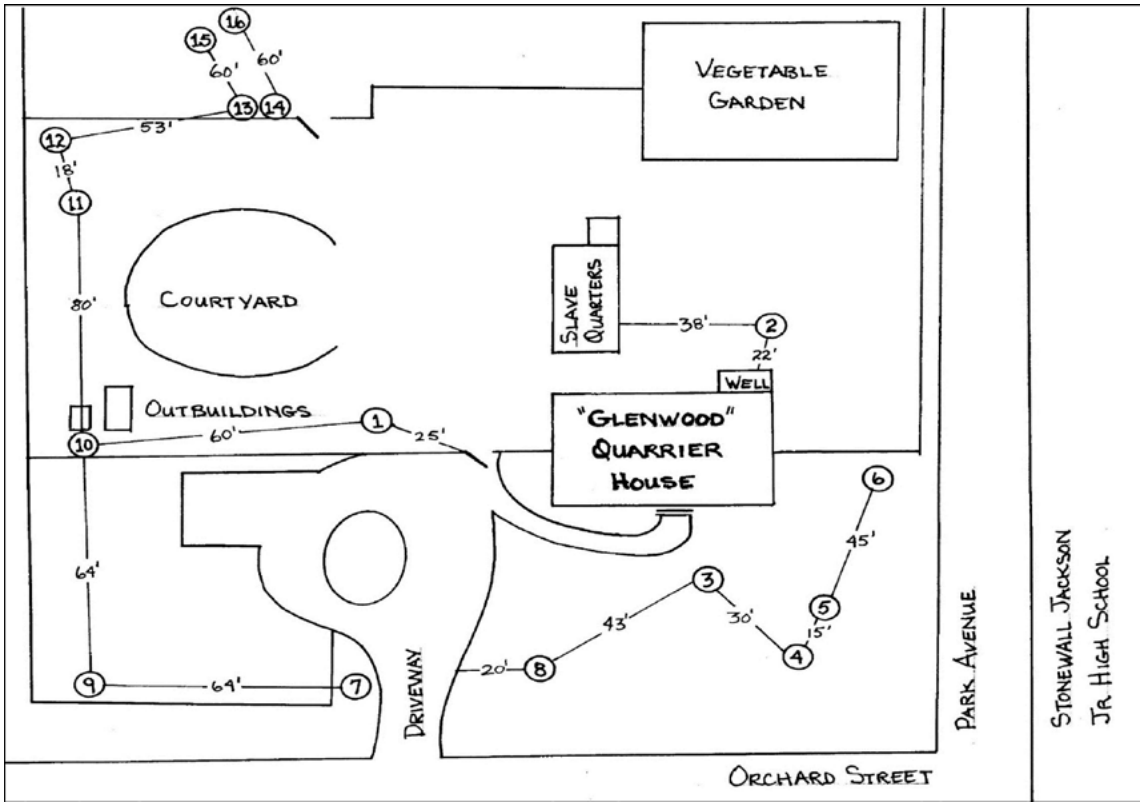

Figure 3.—Schematic of the Glenwood Estate with the American elm trees (1-16) numerically designated.

Materials and Methods

When we began our chemical injection treatment in 1984, there were 16 American elms on the Glenwood property. A few elm stumps were evident, suggesting some trees had already died, presumably from DED. We previously had purchased an elm injection system from the Elm Research Institute (ERI) in Keene, NH, for use at WVU to treat campus elms. We have used this same system and protocol over the 34 years of our involvement at Glenwood. At the time of first treatment in 1984, there was one tree on the property that was displaying the early symptoms of the disease. That year we treated the symptomatic elm and the other healthy elms. In 1984, the trees ranged in diameter from 30.5 cm to 81.3 cm and were distributed throughout the property (Fig. 3). After 32 years, tree diameters ranged from 53.3 cm to 182.9 cm.

The ERI injection protocol involved connecting the injection heads to each other by a Tygon® tubing manifold, drilling injection holes at 10-20 cm intervals on the tree root flares, and then firmly inserting each injection head into a hole (Fig. 4). Arbotect 20-S was chosen for treatment and has been applied in the spring every third year with the exception of a 4-year interval between the 2012 and 2016 treatment. The rate of application was 13.94 ml of chemical per cm of tree diameter. Arbotect was diluted in water so that trees received 32-48 liters of the diluted fungicide depending on their diameter. Uptake of this solution varied for each tree and treatment period, presumably depending on the rate of transpiration. Since we were not resident on the site, Clark Haynes, a forest pathologist with the WVDA, volunteered to periodically check the health of the trees each season.

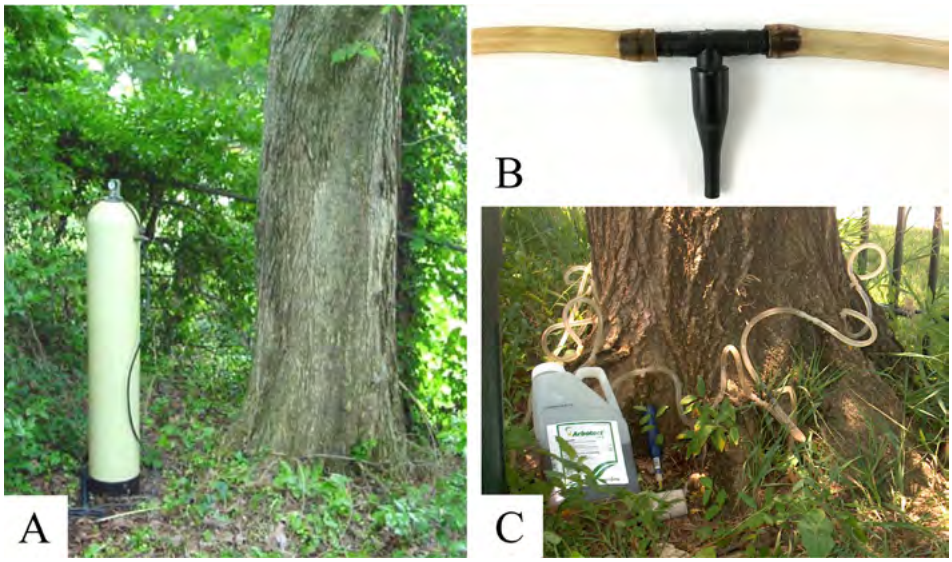

Figure 4.—Elm Research Institute (ERI) injection system: A) fungicide reservoir tank; B) Tygon tubing and injection head; C) injection heads installed in holes along root flare (photo courtesy of VanBooven Tree Care).

Results and Discussion

The chemotherapy for DED was really its infancy when we began the Arbotect injection treatments at Glenwood. Like the technology, our experience with the success or failure of treatment also was very limited. However, over the intervening years it became increasing evident that the treatment had great potential and provided a successful way to manage high value trees. While potential for root-graft transmission of the fungus existed, the treatments eliminated its expression. Of the original 16 elms, we lost four trees over the 32-year period. The original infected tree when we began treatment in 1985 died and was removed soon after. Two trees were removed in 1991 and 2007. We were never advised as to the reason. One additional elm was lost in 2010 when Charleston experienced a devastating windstorm. Overall, the trees have remained healthy and have grown significantly. Their growth is particular noteworthy when observed in an aerial photograph of the Glenwood Estate and the surrounding neighborhood captured by Google Earth (Fig. 5).

The Glenwood Estate remains as a remarkable remnant of the past in a city that grew up around it. Without the populations of majestic American elms that reside there, much of the ambiance of the property would be lost.

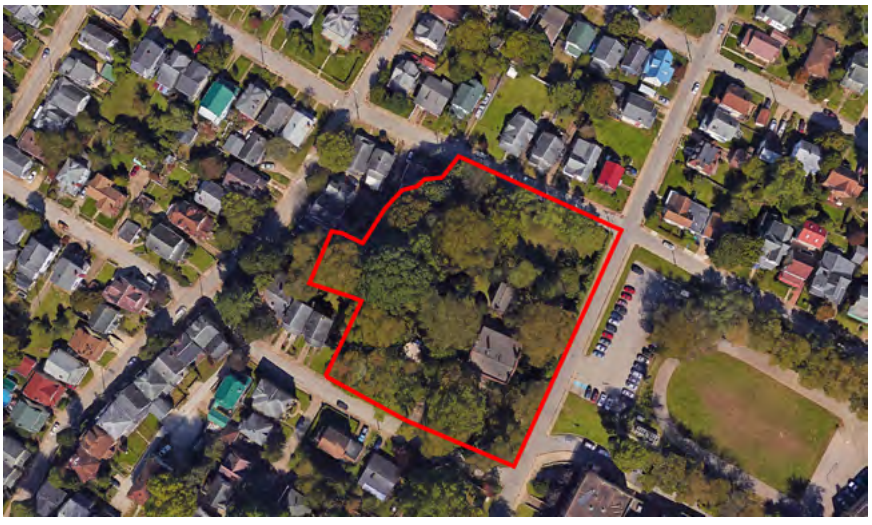

Figure 5.—Aerial view of the Glenwood Estate; property boundary is outlined in red. Google Map.

Literature Cited

Calwell, B. 2014. **Glenwood: a treasure trove of history.** Wonderful West Virginia Magazine. November 2014: 10-12.

Haugen, L.; Stennis, M. 1999. **Fungicide injection to control Dutch elm disease: understanding the options.** Plant Diagnosticians Quarterly. 20(2): 29-38.

NOVEL INSIGHTS INTO THE ELM YELLOWS PHYTOPLASMA GENOME AND INTO THE METAGENOME OF ELM YELLOWS-INFECTED ELMS

Cristina Rosa, Paolo Margaria, Scott M. Geib, and Erin D. Scully[1]

Abtsract.—In North America, American elms were historically present throughout the northeastern United States and southeastern Canada. The longevity of these trees, their resistance to the harsh urban environment, and their aesthetics led to their wide use in landscaping and streetscaping over several decades. American elms were one of most cultivated plants in the United States until the arrival of Dutch elm disease (DED) and elm yellows disease (EY). EY epidemics have killed large numbers of elm trees in the northeastern United States beginning in the 1940s. Since then, the disease has gradually been spreading to the southern and western regions of the United States while remaining endemic in the Northeast. Today EY, together with DED, is responsible for the death of most of the American species of elm trees, including (*Ulmus americana* (L.), *U. rubra (*Muh.), *U. alata* (Michx.), *U. crassifolia* (Nutt.) *U. serotina* (Sarg)) and of some of their natural hybrids (i.e. *U. pumila* × *rubra*).

We performed next-generation sequencing on EY-infected elm trees to discover EY effector genes involved in plant-phytoplasma interactions and to survey the metagenome of the infected elms. This research is a basic step to understand how EY infection shapes the elm microbial communities and, in the long term, will lead to a better understanding of the pathogenesis of EY infection in elm and the interactions between EY and its leafhopper vectors.

Introduction

Elm yellows (EY) epidemics have killed large numbers of elm trees in the northeastern United States beginning in the 1940s (Carter and Carter 1974, Lanier et al. 1988, Sinclair 1972). EY is an important yet underestimated disease that kills infected trees in 1 to 3 years, depending on the size of the tree (Marcone 2016). Some Asian and European elm species are variably resistant to EY, which led to the hypothesis that the causative agent of EY originated in Europe or Asia. In fact, on those continents, the disease manifests with much milder symptoms, and interestingly, European elms grown in North America have not been found to be naturally infected by EY (Sinclair 1981). Areas afflicted with EY in North America include Canada (Niagara peninsula in Ontario since 1984; Matteoni and Sinclair 1989) and the United States, with presence in approximate latitudes 32 to 46° N and longitudes 71 to 97° W. The states where EY is endemic today include Alabama, Arkansas, Georgia, Iowa, Illinois, Indiana, Kansas, Kentucky, Massachusetts, Minnesota, Mississippi, Missouri, Nebraska, New Jersey, New York, Ohio, Oklahoma, Pennsylvania, Tennessee, West Virginia (CABI 1975) and North Dakota (Stack and Freeman 1988).

The pathogens of EY are wall-less bacteria known as phytoplasmas (Pisi et al. 1981, Wilson et al. 1972), and are vectored by leafhoppers (Baker 1948, 1949). Identified EY vectors include

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Scaphoideus luteolus (Van Duzee) (Barnett 1977); the meadow spittlebug *Philaenus spumarius* (L.); the leafhopper *Allygidius atomarius* (Fabricius); and, more recently, the spittlebug (Cercopidae) *Lepyronia quadrangularis* (Say); and a leafhopper in the genus *Latalus* (Cicadellidae: Deltocephalinae) (Rosa et al. 2014). Adult leafhoppers are widespread geographically and are active from early summer until the first frost in autumn. In temperate regions, leafhoppers overwinter as eggs on elm bark and undergo five instars before molting to adults. Development from first instar to adulthood occurs over a period of about 40 days. Leafhopper nymphs are believed to acquire the EY phytoplasmas in mid-June and begin transmitting it after the incubation period, approximately 3 weeks later from mid-July to September (Sinclair et al. 1976). About 7 weeks post-inoculation, infected trees can serve as reservoirs of new infections. Temperatures below –15 ˚F limit the dissemination of both the vectors and the disease. EY phytoplasmas are vectored exclusively by insects and are obligate pathogens of both their host plants and insects.

Once phytoplasmas are introduced to their host plants, they infect sieve elements in phloem tissues of the elms (Braun and Sinclair 1976). After infection, symptoms typically manifest 3 months post inoculation in young plants and up to 9 months in older trees.[2] Eventually, during the late summer, symptoms such as yellowing of the leaves appear and necrosis of the root system, phloem, and xylem tissues become especially pronounced. Since infected trees are impossible to save, the only solution is to remove them as soon as possible. One extension-type publication claims that EY can be transmitted via root grafting,[3] thus, the root systems of infected trees should also be quickly isolated from the roots of neighboring trees to prevent transmission. Usually the EY population is higher in petioles of brooms of live plants than in dead plants, since the pathogen is an obligate parasite. EY overwinters in the roots of elms, moving into the upper branches in the spring (Braun and Sinclair 1976).

Phytoplasmas are grouped on the basis of their 16S rDNA gene sequence into several ribosomal groups. Strains within the various ribosomal groups are often sub-grouped based on geographical origin and on sequencing of other genes (e.g., elongation factor Tu: *TuF*, variable membrane protein 1: *vmp1*). EY of the reference group *Candidatus* Phytoplasma ulmi [16SrV-A] (Lee et al. 2004) is classified in the 16SrV-A lineage, and, based on host specificity, represents a single species. However, there are three known strains of EY: common, Illinois, and European (OEPP/EPPO 1979). It is possible that other strains exist and that other phytoplasmas, such as aster yellows and clover proliferation, may be causing EY-like symptoms (Jacobs et al. 2003). Phytoplasmas secrete effectors directly into the host cytoplasm of sieve cells via the Sec-dependent protein translocation pathway, and the effectors then unload from the phloem to target other plant cells by symplastic transport (Bai et al. 2009, Hoshi et al. 2009, Sugio et al. 2011b). However, unlike other canonical plant pathogens, genes for the type III and type IV secretion systems and pili are noticeably absent in phytoplasmas, probably because phytoplasmas are introduced into cells directly by their insect vectors during feeding (Kakizawa et al. 2010). Consequently, identification and characterization of phytoplasma effectors are paramount for understanding the processes of host colonization and pathogenicity. Infection with phytoplasmas induces notable changes in plant hormonal balance; specifically, potato purple top phytoplasma causes the reduction of gibberellic acid in tomatoes (Ding et al. 2013) while *Ca*. Phytoplasma mali infection in apple trees stimulates production of plant volatiles that attract insect vectors (Mayer et al. 2008a, 2008b). Furthermore, *Ca*. Phytoplasma asteris effectors interfere with the jasmonic acid (JA) defense pathway (Sugio et al. 2011a) in *Arabidopsis*

[2] Personal communication from Gary W. Moorman, Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University.

[3] Personal communication from Wayne Sinclair, Cornell University.

plants. These observations suggest that phytoplasmas are adept at manipulating plant-based herbivore defense pathways, allowing insect vectors to feed on host plants for extended periods of time and promoting successful pathogen transmission. Consistent with this hypothesis, it was observed that *Nicotiana attenuata* plants deficient in the JA pathway are more damaged by leafhoppers (Kallenbach et al. 2012). Only four phytoplasma genomes are fully available: two strains belonging to the 16Sr-I group (*Ca.* Phytoplasma asteris; Bai et al. 2006, Oshima et al. 2004); one strain of the 16Sr –X group (*Ca*. P. mali; Kube et al. 2008); and one strain of *Ca.* P. australiense (Tran-Nguyen et al. 2006), related to 16SrXII group. No genome of phytoplasmas belonging to the 16SrV-A lineage have been sequenced yet.

Here, we report the identification and annotation of genome fragments of *Candidatus* Phytoplasma ulmi that include putative bacterial effectors and preliminary observations regarding the composition of the microbial community present on EY infected elms.

Methods

Sample Collection, DNA Extraction and Sequencing, and Metagenomics Analysis

Two elms trees infected with EY were found on the Pennsylvania State University campus (40˚48.408'N, 77˚52.208'W, University Park, PA) and used as sources of plant materials. While the specific genealogy of the trees is not known, one tree resembles an American elm, *Ulmus americana* (L.), and the other a red elm, *U. rubra (*Muh.).

Fifty grams of leaf midribs and phloem from twigs were processed from each of the trees and were used to perform separate total DNA extractions, as in Ahrens and Seemüller (1992), by using CTAB extraction buffer and by adding a partial ultracentrifugation enrichment. The ratio of host DNA to phytoplasma DNA was measured by quantitative real time PCR (qRT-PCR) using the Quantstudio 3D digital PCR System (Applied Biosystems®, Foster City, CA); DNA concentration and quality was quantified by Nanodrop spectrophotometer (Fisher Scientific) and assessed by the Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA).

Standard Illumina MiSeq long-insert paired libraries were prepared from the two DNA samples at the Huck Institutes genomics core facility, Pennsylvania State University, University Park, PA. DNA was sequenced on the Illumina MiSeq, generating approximately 17.8 million 300 × 300 nt paired-end reads with fragment lengths of 500 nt (5.3 Gb). Reads were trimmed to remove residual adapter sequences and low quality bases using Trimmomatic (version 0.32) with the following options: SLIDINGWINDOW:4:15 MINLEN:150, and ILLUMINACLIP:TruSeq3-SE (http://www.usadellab.org/cms/?page=trimmomatic). Filtered reads were uploaded to MG-RAST (Meyer et al. 2008) for taxonomic and functional classification. rRNAs were identified using RNAmmer (Lagesen et al. 2007) and taxonomically classified using the Ribosomal Database Project (RDP) classifier tool (Wang et al. 2007) with an 80 percent confidence threshold for taxonomic classifications. Putative coding regions were predicted using Prokka (Seemann 2014) and were functionally classified via blastp (Altschul et al. 1997) comparisons to the COG (Tatusov et al. 2000), SEED (Mitra et al. 2011), and the nonredundant (NR) protein databases with an evalue threshold of 10^{-5}. Putative taxonomies of protein coding reads were predicted by blastp comparisons to the NR protein database and MEGAN's least common ancestor algorithm (Huson et al. 2007). Reads coding for effector proteins were identified via blastp searches using reads taxonomically classified as originating from Tenericutes as queries and a custom database containing other previously identified phytoplasma effectors. Gene ontology terms for reads assigned to phylum Tenericutes were computed using Blast2GO (Conesa et al. 2005).

Table 1.—Quality and annotation metrics from shotgun metagenomic sequencing from DNA collected from EY infected elm trees

Number of paired-end reads sequenced	17,897,952 (10.48 Gb)
Number of reads that passed QC	15,559,395 (9.32 Gb)
Number of reads with predicted proteins	11,611,550 (6.96 Gb)
Number of reads with predicted rRNAs	36,260 (22 Mb)
Number of protein coding reads from bacteria	17,333 (10.58 Mb)
Number of protein coding reads from fungi	21,588 (12.95 Mb)
Number of protein coding reads from viruses	61,806 (37.09 Mb)

Table 2.—Abundance of retroviral sequences found in EY infected trees and their classification based on BlastP, GenBank

Sequenced GenBank Annotation	Abundance (number of reads)
Petunia vein clearing virus	682
Ambrosia asymptomatic virus 2 UKM-2007	30
Pelargonium vein banding virus	19

Retrovirus Identification and Characterization

Since multiple retroviruses were found to be integrated into the elm genome, specific primers were designed to amplify the retrovirus sequences of two of the most highly represented viruses, namely a *Petunia vein clearing* hypothetical *virus* and hypothetical *Ambrosia symptomatic virus*. These primers were used to re-amplify the *in silico* assembled viral sequences from the original trees, and to construct larger viral contigs by genome walking. All PCR generated products were sequenced by Sanger sequencing at the Penn State Genomic Core Facility. Additional DNA samples obtained from eight elm trees grown at the U.S. Forest Service facility, in Delaware, Ohio, were screened for the presence of the two retroviral sequences.

Results

DNA Sequencing and Metagenomics Analysis

Approximately 10.48 Gb paired-end MiSeq reads were sequenced from tissues collected from EY infected elm trees, of which about 9.32 Gb passed all quality filters. Most of these reads (~7 Gb) contained predicted coding regions while rRNA sequences accounted for 22Mb. Although most of the reads originated from the host tree, reads originating from fungi, viruses, and bacteria were also readily identified. Viral proteins accounted for most of the microbial coding regions included in the elm metagenome (39 Mb), while fungal and bacterial proteins accounted for 13 Mb and 11Mb, respectively (See Table 1). Bacterial protein-coding regions were classified to 24 different phyla with Proteobacteria, Tenericutes, and Firmicutes being the most highly represented. Notably, the vast majority of the protein coding reads assigned to the phylum Tenericutes had highest scoring blastp matches to proteins from other phytoplasma species. Fungal protein coding regions were classified to 15 different orders with the Eurotiomycetes, Dothideomycetes, and Leotiomycetes as the most highly represented orders, while viruses were almost exclusively assigned to the family Microviridae (bacteriophages, see Fig. 1). Further analyses of the viral sequences after exclusion of bacteriophages identified pararetroviral sequences with high sequence similarities to *Petunia vein clearing virus*, *Ambrosia asymptomatic virus* 2 UKM-2007 and *Pelargonium vein banding virus* (Table 2). We were able to confirm that sequences belonging to two of these three viruses were present, in variable combinations, not only in the DNA extracted from the original tree tissue used for this analysis,

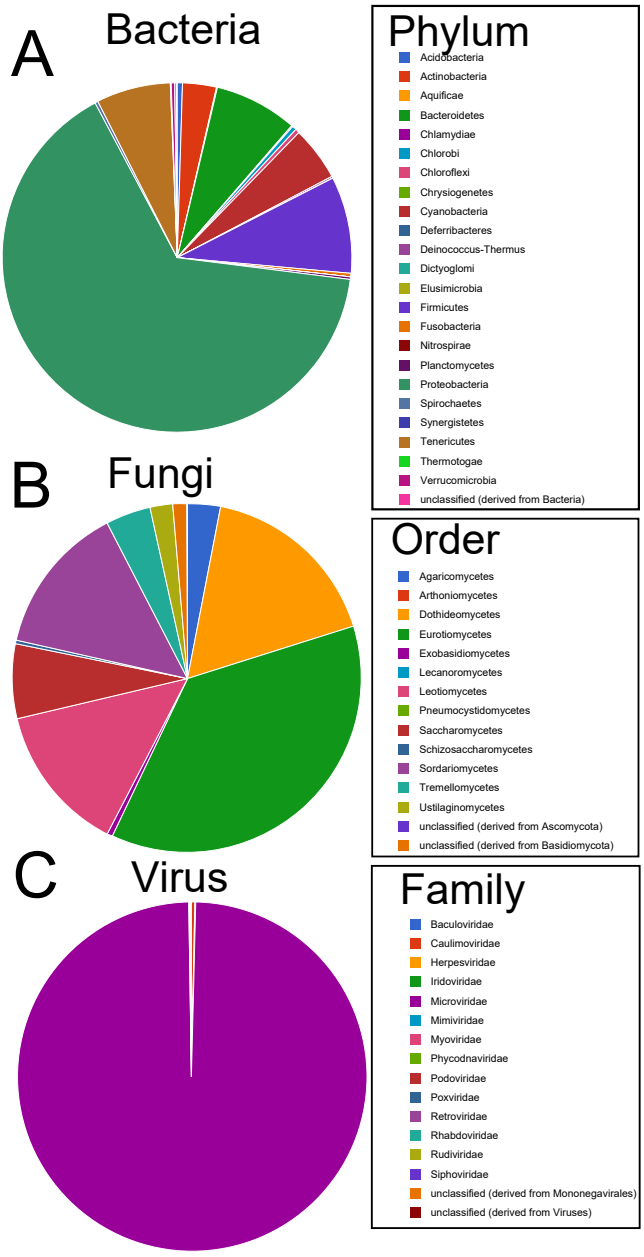

Figure 1.—Taxonomic assignments of predicted protein coding reads from a) bacteria, b) fungi, and c) viruses. Taxonomic assignments of reads predicted to code for proteins were identified via blastp searches to the nonredudant protein database and LCA classification using MEGAN. Bacteria were classified to phylum level, fungi to order level, and viruses to family level. Bacterial reads were classified to 24 different phyla with Proteobacteria, Tenericutes, and Firmicutes being the most highly represented. Fungal reads were classified to 15 different orders with the Eurotiomycetes, Dothideomycetes, and Leotiomycetes as the most represented orders while viruses were almost exclusively assigned to the family Microviridae.

but also in DNA extracted from elm trees collected from another field site in Ohio, suggesting that these viruses are commonly integrated in elm trees, as for other plants.

With regard to rRNA classification, 18 reads containing 16s rRNAs were predicted to originate from Tenericutes while 11 rRNAs were assigned to Burkholderiaceae, and five to Enterobacteriaceae. Other bacterial families detected included Flavobacteriaceae and Cytophagaceae (Fig. 2). Although coding regions containing highest scoring blastp matches to fungi were identified, no fungal rRNAs were identified.

Approximately 850 reads originating from phylum Tenericutes were functionally classified using the SEED database (http://www.theseed.org/). Functional categories including protein metabolism, clustering-based subsystems, carbohydrates, RNA metabolism, and DNA metabolism were highly represented (Fig. 3). The EY key metabolic functions included DNA replication, tRNA aminoacylation for protein translation, nucleobase containing compound

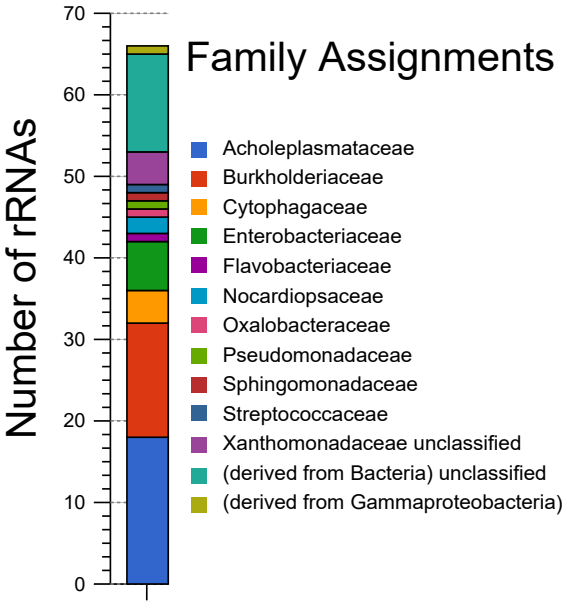

Figure 2.—Class level assignments of bacterial rRNAs detected in EY infected elm tissue. Approximately 65 reads containing bacterial rRNAs were identified using RNAmmer and taxonomically classified to family level using RDP classifier with an 80% confidence threshold. Eighteen rRNAs predicted to originate from Tenericutes, 11 rRNAs predicted to originate from Burkholderiaceae, and five rRNAs predicted to originate from Enterobacteriaceae were identified. Other families detected included Flavobacteriaceae and Cytophagaceae. No rRNAs from fungi or other eukaryotic microbes were identified in this dataset.

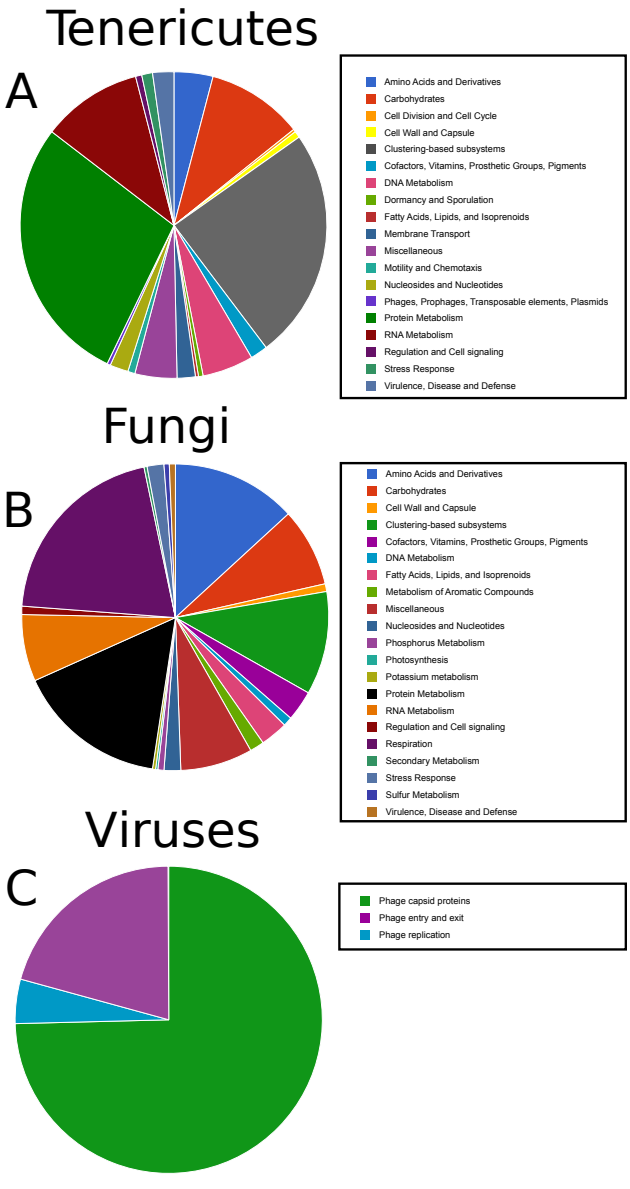

Figure 3.—SEED subsystems assignments for reads assigned to phylum Tenericutes, as Fungi, and as Viruses The main functional categories associated with A) Tenericutes were protein metabolism, clustering-based subsystems, carbohydrates, RNA metabolism, and DNA metabolism. Also, there were a little over 700 reads that were classified as Tenericutes and had similarities to proteins in SEED. For fungi, B), there were 340 reads classified as fungi that had similarity to proteins in SEED.

Again, protein metabolism, clustering based subsystems, and carbohydrates were three of the most prominent categories. In addition, the categories amino acids and derivatives, respiration, cofactors, vitamins, prosthetic groups, pigments, and acids, lipids, and isoprenoids were also well represented.

For viruses, C), there were 28,230 reads classified as virus that had similarity to proteins in seed. The majority of these were capsid proteins, with small numbers of entry and exit and replication proteins identified.

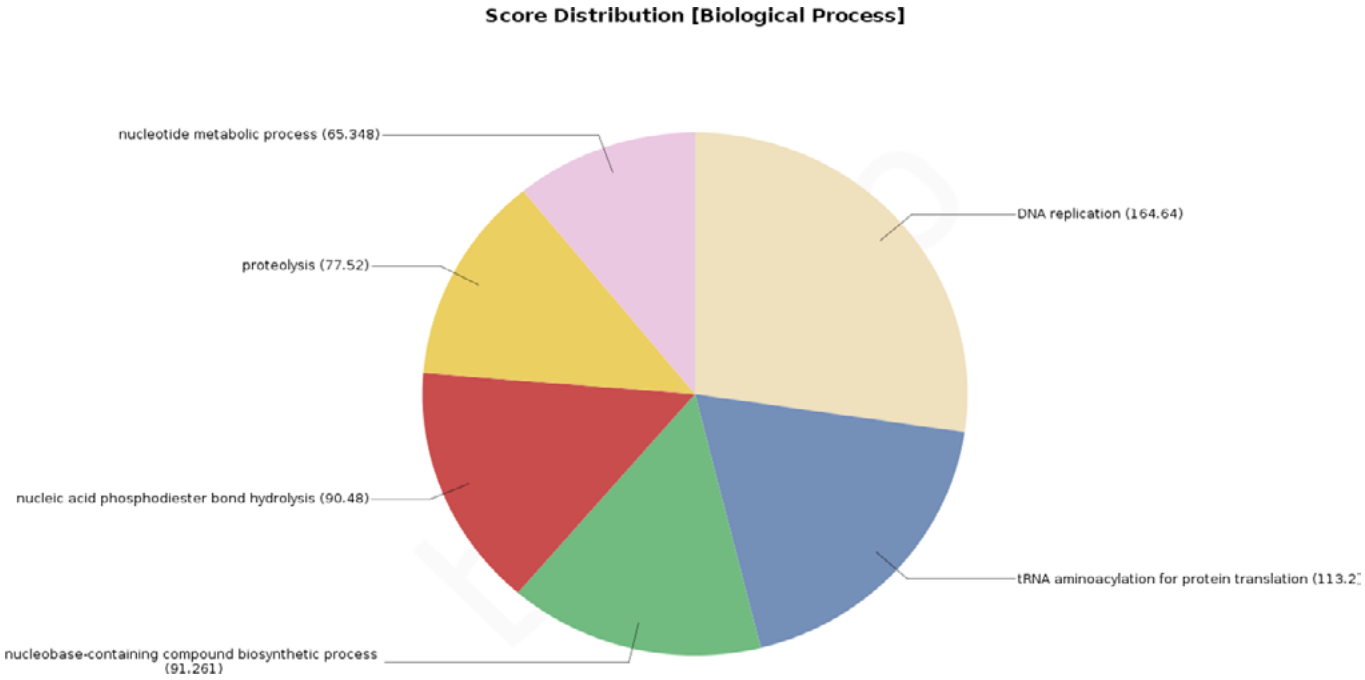

Figure 4:—Score Distribution of EY Biological Processes. The main GO biological process identified for EY were DNA replication, tRNA aminoacylation for protein translation, nucleobase containing compound biosynthetic process, nucleic acid phosphodiester bond hydrolysis, proteolysis and nucleotide metabolic process.

biosynthetic process, nucleic acid phosphodiester bond hydrolysis, proteolysis and nucleotide metabolic process (Fig. 4). EY molecular functions were: ATP binding, metal ion binding, nuclease activity, RNA binding, nucleotidyltransferase activity, DNA binding, ligase activity, and nucleoside-triphosphatase activity (Fig. 5). In addition, Table 3 contains the reads derived from phytoplasmas with their highest scoring blast match, and with the number of reads for each annotation. Using this information, a number of reads coding for putative effectors were positively identified including: endo-beta-1,4-glucanase (break down plant cell walls), protein hupB (siderophore), endopeptidase la, hemolysin channel proteins, hemolysin, ABC maltose transport system, ABC sugar transporters, spermidine/putrescine, ABC transporter permease, transcriptional inducers, and repressors of HrcA heat shock proteins. Two components of the Sec transport system were also readily identified, SecY and SecA.

In fungi, functional catagories corresponding to protein metabolism, clustering based subsystems, and carbohydrates were also three of the most prominent categories while other categories such as amino acids and derivatives, respiration, cofactors, vitamins, prosthetic groups, pigments, fatty acids, lipids, and isoprenoids were also well represented. Viral proteins were mainly capsid proteins, while comparatively smaller numbers of entry and exit and replication proteins were also identified (Fig. 3).

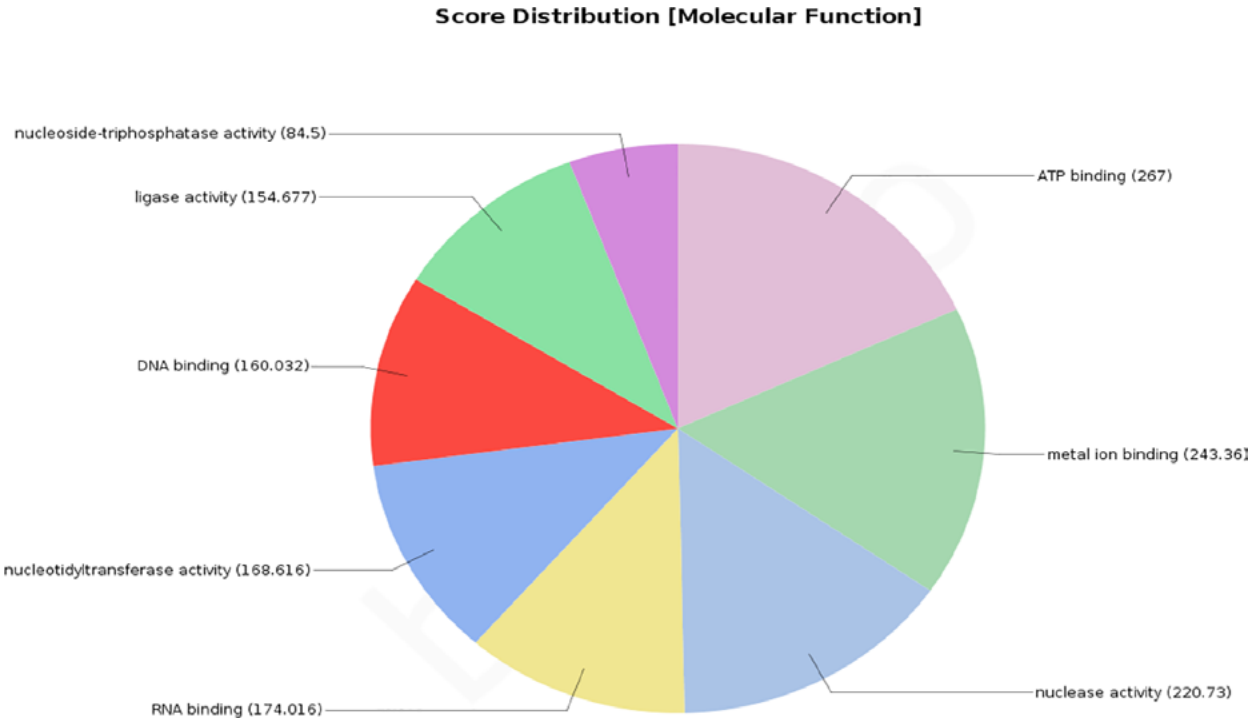

Figure 5.—EY score distribution of molecular functions. EY GO molecular functions were ATP binding, metal ion binding, nuclease activity, RNA binding, nucleotidyltransferase activity, DNA binding, ligase activity and nucleoside-triphosphatase activity.

Table 3.—Phytoplasma reads, their highest scoring blast match, and number of reads for each annotation

Number of reads	Annotation
138	hypothetical protein
30	DNA primase
25	replicative DNA helicase
25	DNA polymerase III subunit alpha
17	DNA double-strand break repair Rad50 ATPase
16	AAA+ ATPase
14	Thymidylate kinase
14	PolC-type DNA polymerase
14	phage-Associated protein
12	DNA helicase
12	AAA+ ATPase, partial
11	exonuclease VII large subunit
11	DNA polymerase III, alpha subunit, Gram-positive
10	conserved hypothetical protein
9	MULTISPECIES: endonuclease
9	endonuclease
9	conserved hypothetical protein, partial sequence,
9	Cell division protein ftsH-like protein

continued

Number of reads	Annotation
9	ATP-dependent DNA helicase
8	zinc ABC transporter permease
8	protein hupB
8	cell division protein FtsH
7	phage-associated protein
7	hypothetical protein S284_00240
7	DNA primase, partial CDS, partial
6	MULTISPECIES: DNA primase
6	hypothetical protein, partial sequence, partial
6	DNA-directed RNA polymerase specialized sigma
6	dihydrolipoyl dehydrogenase
6	ATPase
6	50S ribosomal protein L9
5	valine—tRNA ligase
5	tRNA uridine-5-carboxymethylaminomethyl(34) synthesis
5	sugar ABC transporter permease
5	leucine—tRNA ligase
5	Holliday junction resolvase RecU
5	endopeptidase La
5	DNA gyrase subunit A
5	DNA-directed RNA polymerase subunit beta
5	cysteine—tRNA ligase
4	tRNA (adenosine(37)-N6)-threonylcarbamoyltransferase
4	MULTISPECIES: hypothetical protein
4	methionine—tRNA ligase
4	MBL fold metallo-hydrolase
4	DNA replication protein
4	DNA polymerase III alpha subunit, partial CDS, partial
4	conserved hypothetical protein, partial CDS, partial
4	asparagine—tRNA ligase
4	alanine—tRNA ligase
3	tRNA(Ile)-lysidine synthetase
3	translation initiation factor IF-2
3	spermidine/putrescine ABC transporter permease
3	rRNA (cytidine-2′-O-)-methyltransferase
3	ribosomal RNA small subunit methyltransferase
3	ribonuclease Y
3	peptide chain release factor 2
3	lipoprotein ABC transporter ATP-binding protein
3	histidine—tRNA ligase
3	glutamine—tRNA ligase
3	dTMP kinase

continued

Number of reads	Annotation
3	DNA-directed RNA polymerase subunit beta'
3	DNA-binding protein
3	deoxyribonuclease IV
3	chromosomal replication initiator protein DnaA
3	channel protein, hemolysin III family
3	cation transport ATPase
3	ABC-type maltose transport system, permease protein
3	50S ribosomal protein L3
3	23S rRNA (guanosine(2251)-2'-O)-methyltransferase
2	tRNA (guanosine(37)-N1)-methyltransferase TrmD
2	transcription elongation factor GreA
2	threonine—tRNA ligase
2	sugar ABC transporter substrate-binding protein
2	spermidine/putrescine ABC transporter ATP-binding
2	signal recognition particle protein
2	ribonuclease J
2	pyruvate dehydrogenase (acetyl-transferring) E1
2	primosomal protein N'
2	preprotein translocase subunit SecE
2	PolC-type DNA polymerase III
2	phosphohydrolase
2	peptide chain release factor 1
2	MULTISPECIES: ribosome-recycling factor
2	multidrug ABC transporter permease
2	metallo-beta-lactamase superfamily protein, partial
2	isoleucine—tRNA ligase
2	hypothetical protein S284_01810
2	hypothetical protein S284_01080
2	GTP-binding protein YchF
2	glucose inhibited division protein A, partial sequence,
2	glucose-6-phosphate isomerase
2	exopolyphosphatase
2	excinuclease ABC subunit A
2	elongation factor Ts
2	DNA topoisomerase I
2	DNA polymerase III subunit beta
2	DNA polymerase III, delta prime subunit
2	DNA polymerase III alpha subunit, partial sequence,
2	DNA ligase (NAD(+)) LigA
2	DNA-formamidopyrimidine glycosylase
2	DNA-directed RNA polymerase subunit delta
2	DNA-directed RNA polymerase sigma-70 factor

continued

Number of reads	Annotation
2	diadenosine tetraphosphate hydrolase
2	CTP synthetase
2	copy number control protein (plasmid)
2	conserved hypothetical protein, phage-associated protein
2	class 1b ribonucleoside-diphosphate reductase
2	ATP-dependent zinc metalloprotease FtsH
2	Arginyl-tRNA synthetase, partial sequence, partial
2	adenylate kinase
2	acetate kinase
2	ABC transporter ATP-binding protein
2	50S ribosomal protein L4
2	50S ribosomal protein L35
2	50S ribosomal protein L28
2	50S ribosomal protein L20P, partial CDS, partial
2	50S ribosomal protein L16
2	2,3-bisphosphoglycerate-independent phosphoglycerate
1	YihA family ribosome biogenesis GTP-binding protein
1	Xaa-Pro aminopeptidase, partial sequence, partial
1	Valyl-tRNA synthetase
1	uracil-DNA glycosylase
1	tyrosine—tRNA ligase
1	type I methionyl aminopeptidase
1	type I glyceraldehyde-3-phosphate dehydrogenase
1	tryptophan--tRNA ligase
1	tRNA pseudouridine synthase B
1	tRNA (adenosine(37)-N6)-dimethylallyltransferase
1	triose-phosphate isomerase
1	trigger factor (FKBP-type peptidyl-prolyl cis-trans
1	trigger factor
1	transcription elongation factor NusA, partial sequence,
1	thymidylate synthase
1	sugar permease
1	sugar ABC transporter ATP-binding protein
1	Spermidine/putrescine-binding periplasmic protein
1	sodium transporter
1	site-specific integrase
1	serine protease
1	segregation protein B
1	SAM-dependent methyltransferase
1	rRNA maturation RNase YbeY
1	RNA polymerase sigma factor RpoD
1	ribosome biogenesis GTPase RsgA

continued

Number of reads	Annotation
1	ribosome biogenesis GTPase Der
1	ribosome-binding factor A
1	ribonuclease P protein component
1	Ribonuclease III
1	ribonuclease HIII
1	pyruvate kinase, partial sequence, partial
1	Pyruvate kinase
1	putative endo-1,4-beta-glucanase, partial sequence,
1	pseudouridylate synthase
1	protein translocase component YidC
1	Protein translocase
1	proteasome-activating nucleotidase
1	Prolyl-tRNA synthetase
1	preprotein translocase subunit SecY
1	preprotein translocase subunit SecA
1	Predicted HAD-superfamily hydrolase
1	predicted ATPase AAA-type, contains CbxX/CfqX motif
1	predicted AAA+ ATPAse
1	predicted AAA+ ATPase
1	(p)ppGpp synthetase
1	Phosphoglyceromutase
1	Phosphoglycerate kinase
1	phosphatidylserine decarboxylase
1	phosphatidate cytidylyltransferase
1	phenylalanine--tRNA ligase subunit alpha
1	Phage-Associated Protein
1	peptidyl-tRNA hydrolase
1	peptide transporter
1	peptide ABC transporter substrate-binding protein
1	peptide ABC transporter permease
1	O-methyltransferase
1	nucleotide exchange factor GrpE
1	NAD+ synthetase
1	Na+-driven multidrug efflux pump
1	NADH oxidase
1	MULTISPECIES: transcription elongation factor
1	MULTISPECIES: SsrA-binding protein
1	MULTISPECIES: ribosomal RNA small subunit methyltransferase
1	MULTISPECIES: manganese ABC transporter ATP-binding
1	MULTISPECIES: elongation factor Ts
1	MULTISPECIES: elongation factor 4
1	MULTISPECIES: dTMP kinase

continued

Number of reads	Annotation
1	MULTISPECIES: DNA ligase (NAD(+)) LigA
1	MULTISPECIES: 50S ribosomal protein L33
1	MULTISPECIES: 50S ribosomal protein L28
1	MULTISPECIES: 30S ribosomal protein S2
1	MULTISPECIES: 30S ribosomal protein S15
1	MULTISPECIES: 16S rRNA maturation RNase YbeY
1	Multidrug resistance ABC transporter ATP-binding and
1	multidrug ABC transporter ATP-binding protein
1	molecular chaperone DnaK
1	methionine adenosyltransferase
1	Malate/Na+ symporter
1	malate:citrate symporter
1	lysine—tRNA ligase
1	lipoate—protein ligase
1	kinase
1	inorganic pyrophosphatase
1	hypothetical protein, YrdC-like domain protein
1	hypothetical protein S284_01820
1	hypothetical protein, partial CDS, partial
1	HrcA family transcriptional regulator
1	Holliday junction DNA helicase RuvB
1	Holliday junction DNA helicase RuvA
1	hemolysin
1	Heat-inducible transcription repressor HrcA
1	haloacid dehalogenase
1	guanosine polyphosphate pyrophosphohydrolase
1	GTP pyrophosphokinase
1	GTPase ObgE
1	glycine—tRNA ligase
1	glycerol-3-phosphate acyltransferase
1	Glyceraldehyde-3-phosphate dehydrogenase
1	glutamate—tRNA ligase
1	Glucose-inhibited division protein A, partial sequence,
1	fructose-1,6-bisphosphate aldolase, class II
1	Formamidopyrimidine-DNA glycosylase
1	fatty acid-binding protein DegV
1	Excinuclease ATPase subunit A, partial sequence, partial
1	energy-coupling factor transporter ATP-binding
1	energy-coupling factor transporter ATPase
1	elongation factor Tu
1	elongation factor P
1	elongation factor 4

continued

Number of reads	Annotation
1	DNA-directed RNA polymerase subunit alpha
1	DNA-directed RNA polymerase beta chain, partial sequence,
1	dipeptide transport ATP-binding protein DppF
1	dipeptide/oligopeptide/nickel ABC transporter
1	dimethyladenosine transferase
1	DEAD/DEAH box helicase family protein, SrmB-like
1	cytidine(C)-cytidine(C)-adenosine (A)-adding
1	cobalt ABC transporter ATP-binding protein
1	CMP-binding protein
1	Chromosomal replication initiator protein DnaA, partial
1	CDP-diacylglycerol--serine O-phosphatidyltransferase
1	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase
1	cation uptake P-type ATPase
1	Cation transport ATPase, partial sequence, partial
1	Calcium-translocating P-type ATPase A
1	cadmium-transporting ATPase
1	ATP-dependent Zn protease
1	ATP-dependent zinc metalloprotease FtsH 3
1	ATP-dependent zinc metalloprotease FtsH 2
1	aspartyl-tRNA synthetase, partial sequence, partial
1	aspartate--tRNA ligase
1	arginine--tRNA ligase
1	acyl carrier protein
1	ABC transporter substrate-binding protein
1	AAA+ ATpase
1	6-phosphofructokinase
1	5′-3′ exonuclease
1	50S ribosomal protein L7/L12
1	50S ribosomal protein L6
1	50S ribosomal protein L24
1	50S ribosomal protein L21
1	30S ribosomal protein S8
1	30S ribosomal protein S6
1	30S ribosomal protein S16
1	30S ribosomal protein S15
1	1-acyl-sn-glycerol-3-phosphate acyltransferase
1	16S rRNA pseudouridylate synthase
1	16S rRNA (adenine(1518)-N(6)/adenine(1519)-N(6))-dimethyltransferase

Conclusions

Phytoplasmas can infect about 1,000 different plant species (McCoy et al. 1989). However, despite their importance as plant pathogens, only a handful of phytoplasma genomes have been sequenced. This lack of sequence availability is due to the intrinsic properties of these bacteria that make them particularly challenging to work with. Though phytoplasmas are evolutionarily derived from gram positive ancestors, they lack a cell wall and cannot be cultured in axenic conditions (Firrao et al. 2004). Furthermore, their AT-rich genomes are significantly reduced in size relatively to other bacterial plant pathogens (Marcone and Seemuller 2001, Marcone et al. 1999, Neimark and Kirkpatrick 1993), ranging in size from 300 to 700 Mb. The high richness further impedes genome sequencing efforts as designing specific primers for PCR-based sequencing is very difficult. Adding further to these complications are the presence of large numbers of mobile genetic elements (Kube et al. 2008) and potential mobile units (PMUs) within the genomic DNA that have the potential to reshuffle gene orders (Bai et al. 2006). PMUs are suggested to be mobile elements involved in phytoplasma host switching (Toruno et al. 2010). In addition, many phytoplasmas contain plasmids (Kube et al. 2008, Tran-Nguyen 2006); however, not much is known about their function.

After the arrival of EY at Pennsylvania State University campus (University Park, PA), researchers developed EY detection techniques via a highly specific real time RT-PCR assay (Herath et al. 2010), monitored the EY incidence on the PSU campus during the last 3 years, and determined the seasonal distribution pattern of the phytoplasma in infected trees. Researchers tested more than 1000 elm samples from 471 trees (Herath et al. 2010), and identified two new insect species as EY vectors (Rosa et al. 2014). The next step in our research at Penn State is to offer novel information on EY phytoplasma genome, and especially to identify putative phytoplasma effectors (SAP). Effectors are molecules secreted by the bacteria into the cells of the hosts. SAPs can change flower development and leaf shape and can modify plant-insect interactions, increasing phytoplamsa fitness. For instance, the aster yellows (AY) phytoplasma strain witches' broom (AY-WB) SAP11 is localized in the cell nuclei, deregulates jasmonic acid production, and produces symptoms (witches' broom phenotype). AY-WB has more than 50 effectors (Sugio et al. 2011a).

Based on our preliminary analyses, EY infected trees contain a metagenomics core that includes many bacteria and fungi. The bacteria found belong to families containing plant pathogenic bacteria as well as bacteria associated with plant, soil, and insects.

Data obtained in this study did not allow us to classify the fungi below the order level, but several of the coding regions have highest scoring blastp matches to Dutch elm disease associated fungi. Studying the identity of these fungi could bring some knowledge as to their use as biocontrols against DED.

The elm genome contains many pararetroviral sequences. We don't know if these integrated viruses generate episomal infections, but our tests suggest that the presence and number of pararetroviral sequences could be used as elm phylogenetic tool. Elm phylogeny is complicated and will eventually rely on classification based on key elm genes, but the use of retroviral sequences for classification could be an easier way that could be used until the elm phylogeny is not completely resolved. Many bacteriophage sequences were also found in the EY microbial reads, probably integrated in the plant genome as well as in the genomes of the plant associated microbes. In conclusion, this study represents the first step in the study of EY genome and of the metagenomics community associated with EY infected trees.

Acknowledgments

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Literature Cited

Ahrens, U.; Seemüller, E. 1992. **Detection of DNA of plant pathogenic mycoplasmalike organisms by a polymerase chain reaction that amplifies a sequence of the 16 S rRNA gene.** Phytopathology. 82(8): 828-832.

Altschul, S.F.; Madden, T.L.; Schäffer, A.A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D.J. 1997. **Gapped BLAST and PSI-BLAST: a new generation of protein database search programs.** Nucleic Acids Research. 25(17): 3389-3402.

Bai, X.; Zhang, J.; Ewing, A.; Miller, S.A.; Radek, A.J. [et al.]. 2006. **Living with genome instability: the adaptation of phytoplasmas to diverse environments of their insect and plant hosts.** Journal of Bacteriology. 188(10): 3682-3696.

Bai, X.; Correa, V.R.; Toruño, T.Y.; Ammar, E.D.; Kamoun, S.; Hogenhout, S.A. 2009. **AY-WB phytoplasma secretes a protein that targets plant cell nuclei.** Molecular Plant-Microbe Interactions. 22(1): 18-30.

Baker, W.L. 1948. **Transmission by leaf hoppers of the virus causing phloem necrosis of American elm.** Science. 108(2803): 307-308.

Baker, W.L. 1949. **Studies on the transmission of the virus causing phloem necrosis of American elm, with notes on the biology of its insect vector.** Journal of Economic Entomology. 42(5): 729-732.

Barnett, D.E. 1976. **A revision of the Nearctic species of the genus Scaphoideus (Homoptera: Cicadellidae).** Transactions of the American Entomological Society. 102(4): 485-593.

Braun, E.J.; Sinclair, W.A. 1976. **Histopathology of phloem necrosis in *Ulmus americana*.** Phytopathology. 66: 598-607.

CABI. 1975. **Elm phloem necrosis virus, 2nd edition [distribution map]. Map 107.** In: Distribution maps of plant diseases. Wallingford, UK: CAB International. http://www.cabi.org/dmpd/ (June 6, 2017).

Carter, J.E.; Carter, L.R. 1974. **An urban epiphytotic of phloem necrosis and Dutch elm disease, 1944-1972.** Illinois Natural History Survey Bulletin. 31(4): 112-143.

Conesa, A.; Götz, S.; García-Gómez, J.M.; Terol, J.; Talón, M.; Robles, M. 2005. **Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research.** Bioinformatics. 21(18): 3674-3676.

Ding, Y.; Wei, W.; Wu, W.; Davis, R.E.; Jiang, Y. [et al.]. 2013. **Role of gibberellic acid in tomato defence against potato purple top phytoplasma infection.** Annals of Applied Biology. 162(2): 191-199.

Firrao, G.; Andersen, M.; Bertaccini, A.; Boudon, E.; Bove, J.M. [et al.] 2004. **Candidatus phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects.** International Journal of Systematic and Evolutionary Microbiology. 54(4): 1243-1255.

Herath, P.; Hoover, G.A.; Angelini, E.; Moorman, G.W. 2010. **Detection of elm yellows phytoplasma in elms and insects using real-time PCR.** Plant Disease. 94(11): 1355-1360.

Hoshi, A.; Oshima, K.; Kakizawa, S.; Ishii, Y.; Ozeki, J. [et al.]. 2009. **A unique virulence factor for proliferation and dwarfism in plants identified from a phytopathogenic bacterium.** Proceedings of the National Academy of Sciences. 106(15): 6416-6421.

Huson, D.H.; Auch, A.F.; Qi, J.; Schuster, S.C. 2007. **MEGAN analysis of metagenomic data.** Genome Research. 17(3):377-386.

Jacobs, K.A.; Lee, I.M.; Griffiths, H.M.; Miller Jr, F.D.; Bottner, K.D. 2003. **A new member of the clover proliferation phytoplasma group (16SrVI) associated with elm yellows in Illinois.** Plant Disease. 87(3): 241-246.

Kakizawa, S.; Oshima, K.; Namba, S. 2010. **Functional genomics of phytoplasmas.** In: Weintraub, P.G.; Jones, P., eds. Phytoplasmas: genomes, plant hosts and vectors. Oxford, UK: CAB International: 37-50.

Kallenbach, M., Bonaventure, G., Gilardoni, P.A., Wissgott, A. and Baldwin, I.T. 2012. **Empoasca leafhoppers attack wild tobacco plants in a jasmonate-dependent manner and identify jasmonate mutants in natural populations.** Proceedings of the National Academy of Sciences. 109(24): E1548-E1557.

Kube, M.; Schneider, B.; Kuhl, H.; Dandekar, T.; Heitmann, K.; Migdoll, A.M.; Reinhardt, R.; Seemüller, E. 2008. **The linear chromosome of the plant-pathogenic mycoplasma 'Candidatus Phytoplasma mali'.** BMC Genomics. 9(1): 306.

Lagesen, K.; Hallin, P.; Rødland, E.A.; Stærfeldt, H.H.; Rognes, T.; Ussery, D.W. 2007. **RNAmmer: consistent and rapid annotation of ribosomal RNA genes.** Nucleic Acids Research. 35(9): 3100-3108.

Lanier, G.N.; Schubert, D.C.; Manion, P.D. 1988. **Dutch elm disease and elm yellows in central New York: out of the frying pan into the fire.** Plant Disease. 72(3): 189-194.

Lee, I.-M.; Martini, M.; Marcone, C.; Zhu, S.F. 2004. **Classification of phytoplasma strains in the elm yellows group (16SrV) and proposal of 'Candidatus Phytoplasma ulmi' for the phytoplasma associated with elm yellows.** International Journal of Systemic and Evolutionary Microbiology. 54: 337-347.

Marcone, C. 2016. **Elm yellows: A phytoplasma disease of concern in forest and landscape ecosystems.** Forest Pathology. 47(1): e12324.

Marcone, C.; Neimark, H.; Ragozzino, A.; Lauer, U.; Seemüller, E. 1999. **Chromosome sizes of phytoplasmas composing major phylogenetic groups and subgroups.** Phytopathology. 89(9): 805-810.

Marcone, C.; Seemüller, E. 2001. **A chromosome map of the European stone fruit yellows phytoplasma.** Microbiology. 147(5): 1213-1221.

Matteoni, J.A.; Sinclair, W.A. 1989. **A note on the presence of elm yellows in the Niagara Peninsula.** Phytoprotection. 70(3): 137-139.

Mayer, C.J.; Vilcinskas, A.; Gross, J. 2008a. **Pathogen-induced release of plant allomone manipulates vector insect behavior.** Journal of Chemical Ecology. 34(12):1518-1522.

Mayer, C.J.; Vilcinskas, A.; Gross, J. 2008b. **Phytopathogen lures its insect vector by altering host plant odor.** Journal of Chemical Ecology. 34(8): 1045-1049.

McCoy, R.E.; Caudwell, A.; Chang, C.J. [et al.]. 1989. **Plant diseases associated with mycoplasma-like organisms.** In: Whitcomb, R.F.; Tully, J.G., eds. The mycoplasmas, Vol. 5: Spiroplasmas, Acholeplasmas, and Mycoplasmas of plants and arthropods. New York, NY: Academic Press: 546-640.

Meyer, F.; Paarmann, D.; D'Souza, M.; Olson, R.; Glass, E.M. [et al.]. 2008. **The metagenomics RAST server–a public resource for the automatic phylogenetic and functional analysis of metagenomes.** BMC Bioinformatics. 9(1): 386.

Mitra, S.; Rupek, P.; Richter, D.C.; Urich, T.; Gilbert, J.A. [et al.]. 2011. **Functional analysis of metagenomes and metatranscriptomes using SEED and KEGG. BMC Bioinformatics.** 12(1): S21.

Neimark, H.; Kirkpatrick, B.C. 1993. **Isolation and characterization of full-length chromosomes from non-culturable plant-pathogenic Mycoplasma-like organisms.** Molecular Microbiology. 7(1): 21-28.

OEPP/EPPO [European and Mediterranean Plant Protection Organization]. 1979. **Data sheet on quarantine organisms no. 26. Elm phloem necrosis (mycoplasm).** OEPP/EPPO Bulletin. 9 (2).

OEPP/EPPO [European and Mediterranean Plant Protection Organization]. 1982. **Data sheets on quarantine organisms no. 63. *Ceratocystis ulmi*.** OEPP/EPPO Bulletin. 12 (1).

Oshima, K.; Kakizawa, S.; Nishigawa, H.; Jung, H.Y.; Wei, W. [et al.]. 2004. **Reductive evolution suggested from the complete genome sequence of a plant-pathogenic phytoplasma.** Nature Genetics. 36(1): 27-29.

Pisi, A.; Marani, F.; Bertaccini, A. 1981. **Mycoplasma-like organisms associated with elm witches' broom symptoms.** Phytopathologie Mediterranea. 20(2/3): 189-191.

Rosa, C.; McCarthy, E.; Duong, K.; Hoover, G.; Moorman, G. 2014. **First report of the spittlebug Lepyronia quadrangularis and the leafhopper *Latalus* sp. as vectors of the elm yellows associated phytoplasma, Candidatus phytoplasma ulmi in North America.** Plant Disease. 98(1): 154.

Seemann, T. 2014. **Prokka: rapid prokaryotic genome annotation.** Bioinformatics. 30(14): 2068-99.

Sinclair, W.A. 1972. **Phloem necrosis of American and slippery elms in New York.** Plant Disease Reporter. 56(2): 159-161.

Sinclair, W.A.; Braun, E.J.; Larsen, A.O. 1976. **Update on phloem necrosis of Elms.** Journal of Arboriculture. 2(6): 106-113.

Sinclair, W.A. 1981. **Elm yellows.** In: Stipes, R.J.; Campana, R.J., ed. Compendium of elm diseases. St. Paul, MN: American Phytopathological Society: 25-31.

Stack, R.W.; Freeman, T.P. 1988. **First report of elm yellows in North Dakota.** Plant Disease. 72(10): 912.

Sugio, A.; MacLean, A.M.; Grieve, V.M.; Hogenhout, S.A. 2011a. **Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis.** Proceedings of the National Academy of Sciences. 108(48): E1254-E1263.

Sugio, A.; MacLean, A.M.; Kingdom, H.N.; Grieve, V.M.; Manimekalai, R.; Hogenhout, S.A. 2011b. **Diverse targets of phytoplasma effectors: from plant development to defense against insects.** Annual Review of Phytopathology. 49: 175-195.

Tatusov, R.L.; Galperin, M.Y.; Natale, D.A.; Koonin, E.V. 2000. **The COG database: a tool for genome-scale analysis of protein functions and evolution.** Nucleic Acids Research. 28(1): 33-36.

Toruño, T.Y.; Seruga Musić, M.; Simi, S.; Nicolaisen, M.; Hogenhout, S.A. 2010. **Phytoplasma PMU1 exists as linear chromosomal and circular extrachromosomal elements and has enhanced expression in insect vectors compared with plant hosts.** Molecular Microbiology. 77(6): 1406-1415.

Tran-Nguyen, L.T.T.; Gibb, K.S. 2006. **Extrachromosomal DNA isolated from tomato big bud and Candidatus Phytoplasma australiense phytoplasma strains.** Plasmid. 56(3): 153-166.

Wang, Q.; Garrity, G.M.; Tiedje, J.M.; Cole, J.R. 2007. **Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy.** Applied and Environmental Microbiology. 73(16): 5261-5267.

Wilson, C.L.; Seliskar, C.E.; Krause, C.R. 1972. **Mycoplasmalike bodies associated with elm phloem necrosis.** Phytopathology. 62: 140-143.

ELM YELLOWS: A WIDESPREAD AND OVERLOOKED KILLER OF ELM TREES ACROSS THE UNITED STATES

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Abstract.—The elm yellows phytoplasma (*Candidatus* Phytoplasma ulmi) is a geographically widespread pathogen that poses a significant threat to most native wild elms in North America (*Ulmus americana, U. rubra, U. alata, U. crassifolia,* and *U. serotina*) (Mäurer et al. 1993), as well as to the success of American elm research programs focused on breeding Dutch elm disease tolerance. Despite the advancements of American elm research programs in creating Dutch elm disease-tolerant varieties, elm yellows research has lagged and threatens to undermine the success of breeding programs. Here, we have three goals: 1) to present a general background on elm yellows; 2) to discuss the identification and management of a 2016 elm yellows outbreak in an American elm research plantation in Delaware, OH; and 3) to summarize recent research advancements as well as tools toward identification and management. To date, 9 of 47 trees from the American elm research plantation in Delaware, OH, tested have been confirmed to be infected with phytoplasmas similar to those known to cause elm yellows in other locations. False positives were frequent and improved methods for detecting and identifying the phytoplasma are needed.

Introduction

Elm yellows (EY) is one of the most destructive diseases of elms behind Dutch elm disease (DED), caused by the fungal pathogens *Ophiostoma ulmi* (Buisman) Melin & Nannf. and *O. novo-ulmi* Brasier (Lee et al. 2004, Marcone 2016). Unlike the DED fungal pathogens, which stimulate a defensive response in the tree that clogs xylem tissue, elm yellows is caused by phloem-obligate bacteria called phytoplasmas (in the class Mollicutes), which multiply in the phloem, sieve elements, and disrupt nutrient translocation. Phytoplasmas are classified into groups based on the nucleotide sequence of the 16S rDNA gene. Elm yellows can be caused by a number of phytoplasma groups, including the elm yellows (Group16SrV-A) (Lee et al. 2004), Illinois elm yellows (Group16SrVI-C) (Jacobs et al. 2003), and the aster yellows (Group16SrI) (Lee et al. 1995).

The primary vectors for EY are vascular-feeding insects (Order Hemiptera, including Cicadellidae and Cercopidae families) (Baker 1948). It has been suggested that the phytoplasma may be transmitted between elm trees via root grafts (Sinclair 2000), but experimentation has not been conducted to substantiate this claim. Elms infected with the pathogen exhibit rootlet necrosis followed by degeneration of phloem in the lower trunk, foliar chlorosis, and epinasty (Sinclair 2000). Infected vascular tissue exposed by peeling bark off of a fresh sample may exhibit a butterscotch color as well as the emission of a methyl salicylate (wintergreen) odor (Sinclair 2000).

Likely introduced into North America in the 1800s (Baker 1948, Marcone 2016), EY was first described in Ohio by Swingle (1938) as causing severe decline in American elm (*Ulmus americana* L.) and red elm (*U. rubra* Muhl.). Its initial presence and spread was likely

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underestimated because of the simultaneous occurrence of DED. It is now widespread across much of eastern North America, from Mississippi to southeastern Ontario (Matteroni and Sinclair 1985). Elm yellows has also been reported in parts of Europe: France, Italy, and Serbia (Jović et al. 2011, Marcone 2016, Mittempergher 2000).

Research focusing on breeding DED-tolerant American elms (see Flower et al. 2017, this proceedings) is advancing and efforts are currently underway to transition these trees into the natural environment (Knight et al. 2017, this proceedings). One significant setback on these transition studies are EY outbreaks. An enhanced understanding of the EY pathogen and its vectors is needed, not only for the sake of better understanding of this largely overlooked pathogen, but also to advance DED-resistance work. The objective of this study is to provide a synopsis of identification and mitigation activities conducted in response to an elm yellows outbreak in an American elm plantation in Delaware, OH, during the summer of 2016.

Methods And Materials

Study Site

This work took place in late July 2016 at two plantations in Delaware, OH, named East (4 acres) and Main (5.5 acres). The two plantations are situated approximately 100 yards from each other. General yellowing of established American elm canopies was observed across a portion of the two plantations as well as individual trees in varying stages of dying. Symptomatic trees exhibited EY characteristics including: 1) premature canopy yellowing that was evenly distributed across the canopy; 2) wilt of foliage throughout the canopy; 3) phloem discoloration; and 4) a strong odor of methyl salicylate on a subset of trees. These symptoms developed over the course of 3 weeks.

Genetic Approaches for Identifying the Disturbance Agent

Leaf samples were collected from the upper canopy and phloem samples were taken from branches and twigs of symptomatic and asymptomatic trees. Insects were collected near infected trees using sweep nets and yellow sticky traps. Leaf and phloem tissue were used for identification and DNA analysis. DNA was extracted from the phloem and leaf material using Qiagen DNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands). We used three PCR-based tests for identifying the causal agent: 1) a real-time polymerase chain reaction (rt-PCR) primers designed specifically for *Ca.* Phytoplasma ulmi (Herath et al. 2010); 2), a semi-nested PCR technique by using the phytoplasma universal primer pair P1/P7 that was specifically designed for phytoplasma 16S-23S rRNA genes (Schneider et. al. 1995). The second semi-nested PCR reaction was run using P7 and the reverse complement of the universal phytoplasma primer R16R2 (5'-CGGGGTTTGTACACACCGCCCGTC-3') (Gundersen, 1996). The PCR products were run on a 1.2 percent agarose gel made with 1x Tris-Borate-EDTA (TBE) buffer using ethidium bromide to view the amplified DNA. Based on other phytoplasma sequences, the predicted product size for the second primer pair is 480 base pair.

Third, the semi-nested PCR test of samples with 400-800 bp products was repeated on a larger scale. These PCR products were run on a 1.2 percent TBE gel, then post-stained in 0.0015 percent Nile Blue. Each of the 400-800 bp bands were isolated and gel-purified using GeneClean Spin kit (MPBiomedicals, Solon, OH) and sent to the Plant-Microbe Genomics Facility at Ohio State University for sequencing, using PCR primer P7 as a sequencing primer. The facility uses the 3730 DNA Analyzer from Applied Biosystems, Inc. and BigDye® Terminator Cycle Sequencing chemistry.

Management

To mitigate the damage in the plantations, management consisted of: 1) felling the symptomatic and dead trees for burning; 2) severing possible root grafts by trenching ~100 cm deep to separate the plots within each plantation; and 3) spraying the plantations with the pyrethrin insecticide Talstar® (FMC Corp., Philadelphia PA), to control vectors.

Results and Discussion

During a 3-week period in August 2016, more than 80 trees in both plantations exhibited elm yellows-like symptoms. We promptly removed trees that died as well as those that did not die but were symptomatic. Following removals and the initial wave of yellows-like symptoms in the northern portion of the East Plantation, no further canopy yellowing symptoms were identified.

The real-time PCR approach revealed that three trees tested positive for *Ca*. Phytoplasma ulmi. These three trees consisted of two samples from known EY-positive trees on the Penn State University campus, and one was from an asymptomatic tree in the East Plantation (Table 1). The semi-nested PCR approach yielded products in several trees, both symptomatic and asymptomatic, with products in the 400-800 bp range (Table 1). Despite the primers being designed for phytoplasma specificity, the presence of nine false positives, which as revealed by further sequencing, was apparent. These nine sequences taken from both symptomatic and asymptomatic trees were identified through GenBank as having similarity to sequences of various bacteria genera from soil, skin, and the cloaca of birds. Upon further investigation, it was determined that the phytoplasma PCR primers P7 and R16R2 (reverse compliment) have

Table 1.—Sample results of the symptomatic trees tested using real-time PCR, semi-nested PCR, or sequencing methods. Results are denoted by tree location (OH=Ohio, PA=Pennsylvania); symptomatic (Y=symptomatic, N=not symptomatic); real-time PCR ('+' and '-' denote positive and negative for *Ca*. Phytoplasma ulmi); Semi-nested PCR (Y=400-800 bp bands present, N=400-800 bp bands not present); Sequence (F=Failed [likely due to low DNA concentrations or a mix of different DNAs in the sequence], O=skin/soil bacteria, S=soil bacteria, V=16Sr-V phytoplasma, VI=16SrVI phytoplasma). *denotes offsite control.

			Real-time PCR			
Sample ID	Tree Location	Symptomatic	1st	2nd	Semi-nested PCR	Sequence
1, 33, 35, 36, 38, 41, 43, 45, 46,	OH	N	-	-	Y	F
2, 20, 22, 25, 28, 32, 34, 37, 39, 40, 42, 44, 47	OH	N	-	-	N	
3, 27, 48	OH	Y	-	-	N	
4	OH	Y	-	-	Y	F
5,12,26	OH	Y	-	-	Y	O
6	OH	Y	-		Y	F
7,8	PA	Y	+	+	Y	V
9	OH	N	+	+	Y	O
11*, 19*	OH	N	-		N	
10, 13, 14, 15, 16, 17, 18, 30, 31	OH	Y	-	-	Y	VI
21, 23, 24, 29	OH	N	-	-	Y	S

*denotes offsite control

identical or almost identical sequence in the 16S-23S rDNA genes of other classes of bacteria, not just Mollicutes. For some sequenced samples, more than one DNA with the appropriate size was amplified and purified together during the PCR reaction with the phytoplasma primers, so the sequence could not confirm or deny the presence of phytoplasma. Of the remaining samples yielding clear sequences, one was identified as very closely related to *Ca.* Phytoplasma ulmi, which resides within the elm yellows group (16SrV). This sample was from DNA isolated from the Penn State EY-positive trees. Nine other sequences from symptomatic trees in Delaware, OH, were identical to each other. They were identified through Genbank as most closely related to phytoplasma pathogens in the clover proliferation group (16Sr-VI), similar to the elm phytoplasma 'Arlington Heights' (Genbank Accession AF268893.1) (Table 1).

The discrepancies in identification of the elm yellows phytoplasma between the different analyses indicate that caution should be taken to avoid misidentification of the pathogen. The real-time PCR technique developed for detecting *Ca*. Phytoplasma ulmi may be producing false positives in part because of homology with some related bacterial strains. The Delaware, OH, sample (#9), that repeatedly tested positive for *Ca*. Phytoplasma ulmi using rt-PCR with EY-specific primers, was later sequenced and only found to be positive for soil-borne and bird cloaca-originating bacteria. The semi-nested PCR approach also resulted in several false-positive results from nine of the samples, which were later confirmed to be soil-borne bacteria. The results of this assessment indicate that real-time and the semi-nested PCR approaches should be viewed with healthy skepticism until new primers are designed.

Ongoing research

Because symptom identification is frequently followed by removal, many basic aspects of EY remain understudied. Efforts are currently underway to investigate the seasonal fluctuations of the pathogen within the tree and to assess the susceptibility of different DED-tolerant American elm selections to EY. There is also an ongoing effort to systematically trap insects to quantify the abundance and distribution phytoplasma within the vectors (Rosa et al. 2014). Finally, efforts are underway to reduce identification costs via nested PCR with phytoplasma primers, followed by restriction fragment length polymorphism analysis to assign phytoplasmas to recognized phylogenetic groups.

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References

Baker, W.L. 1948. **Transmission by leaf hoppers of the virus containing phloem necrosis of American elm.** Science. 108: 307-308.

Flower, C.E.; Slavicek, J.M.; Lesser, D.; Eshita, S.; Pinchot, C.C. 2017. **Canopy decline assessments in mature American elm after inoculation with different doses of *Ophiostoma ulmi* and *O. novo-ulmi*.** In: Pinchot, C.C.; Knight, K.S.; Haugen, L.M.; Flower, C.E.; Slavicek, J.M., eds. Proceedings of the American elm restoration workshop 2016; 2016 October 25-27; Lewis Center, OH. Gen. Tech. Rep. NRS-P-174. Newtown Square, PA: U.S. Department of Agriculture, Forest Service Northern Research Station: 24-29.

Gundersen, D.E.; Lee, I.-M. 1996. **Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs.** Phytopathology Mediterranea. 35: 144-151.

Herath, P.; Hoover, G.; Angelini E.; Moorman, G. 2010. **Detection of elm yellows phytoplasma in elms and insects using real-time PCR.** Plant Disease. 94: 1355-1360.

Jacobs, K.A.; Lee, I.-M.; Griffiths, H.M.; Miller, F.D., Jr.; Bottner, K.D. 2003. **A new member of the clover proliferation phytoplasma group (16SrVI) associated with elm yellows in Illinois.** Plant Disease. 87: 241-246.

Jović, J.; Cvrković, T.; Mitrović, M.; Petrović, A.; Krstić, O.; Krnjajić, S. 2011. **Multigene sequence data and genetic diversity among *'Canditatus* Phytoplasma ulmi' strains infecting *Ulmus* spp. in Serbia.** Plant Pathology. 60: 356-368.

Knight, K.S.; Haugen, L.; Pinchot, C.; Schaberg, P.; Slavicek, J.M. 2017. **American elm in restoration plantings: a review.** In: Pinchot, C.C.; Knight, K.S.; Haugen, L.M.; Flower, C.E.; Slavicek, J.M., eds. Proceedings of the American elm restoration workshop 2016; 2016 October 25-27; Lewis Center, OH. Gen. Tech. Rep. NRS-P-174. Newtown Square, PA: U.S. Department of Agriculture, Forest Service Northern Research Station: 133-140.

Lee, I.-M.; Bertaccini, A.; Vibio, M.; Gundersen, D.E.; Davis, R.E. [et al.]. 1995. **Detection and characterization of phytoplasmas associated with diseas in Ulmus and Rubus in northern and central Italy.** Phytopathology Mediterranea. 34: 174-183.

Lee, I.-M.; Martini, M.; Marcone C.; Zhu, S.F. 2004. **Classification of phytoplasma strains in the elm yellows group (16SrV) and proposal of *'Candidatus* Phytoplasma ulmi' for the phytoplasma associated with elm yellows.** International Journal of Systemic and Evolutionary Microbiology. 54: 337-347.

Marcone, C. 2016. **Elm yellows: A phytoplasma disease of concern in forest and landscape ecosystems.** Forest Pathology. 16 p. http://dx.doi.org/10.1111/efp.12324.

Mäurer, R.; Seemüller, E.; Sinclair, W.A. 1993. **Genetic relatedness of mycoplasmalike organisms affecting elm, alder, and ash in Europe and North America.** Phytopathology. 83: 971-976.

Matteoni, J.A.; Sinclair, W.A. 1985. **Role of mycoplasmal disease, ash yellows in decline of white ash in New York state.** Phytopathology. 75: 355-360.

Mittempergher, L. 2000. **Elm yellows in Europe.** In: Dunn E.P., ed. The elms, conservation and disease management. Boston, MA: Kluwer Academic Press: 103-119.

Rosa, C.; McCrathy, E.; Duong, K.; Hoover, G.; Moorman, G. 2014. **First report of the spittlebug *Lepyronia quadrangularis* and the leafhopper *Latalus* sp. as vectors of the elm yellows associated phytoplasma, *Candidatus* Phytoplasma ulmi in North America.** Plant Disease. 98(1): 154.

Schneider, B.; Seemüller, E.; Smart, C.D.; Kirkpatrick, B.C. 1995. **Phylogenic classification of plant pathogenic mycoplasmalike organisms or phytoplasmas.** Molecular and Diagnostic Procedures in Mycoplasmology. 1: 369-380.

Sinclair, W.A. 2000. **Elm yellows in North America.** In: Dunn, C.P., ed. The elms: breeding, conservation and disease management. Boston, MA: Kluwer Academic Press: 121-136.

Swingle, R.W. 1938. **A phloem necrosis of elm.** Phytopathology. 28: 757-759.

AMERICAN ELM ECOLOGY

THE ECOLOGICAL ROLE OF AMERICAN ELM (*ULMUS AMERICANA* L.) IN FLOODPLAIN FORESTS OF NORTHEASTERN NORTH AMERICA

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Abstract.—Before Dutch elm disease, the American elm (*Ulmus americana* L.) was a leading dominant tree species in the better drained parts of floodplain forests where flooding occurs about 1 percent of the time. Although still common in these habitats today, *U. americana* now rarely lives long enough to reach the forest canopy because elm mortality rates increase sharply with tree size. This article reviews the impact that the loss of American elm due to Dutch elm disease has had on floodplain forests, helps provide a clear rationale for restoring this iconic species in the wild, and also provides quantitative benchmarks against which to measure progress. *Fraxinus* L. species are ecologically the most similar species to *U. americana*, but *Fraxinus* trees are now also threatened because of the spread of the invasive emerald ash borer. This new threat to floodplain forests of northeastern North America adds urgency to the need to develop more disease tolerant selections of *U. americana* and plant them into floodplain habitats.

Introduction

The loss of native elms from cities and villages across Europe and North America in the wake of the nonnative Dutch elm disease (DED) and the aesthetic impacts of that loss on the traditional landscape has received much public attention (Campanella 2011, Richens 1983). This cultural loss has been the primary motivation behind past efforts to breed more disease-resistant elms and manage the pathogen and its vectors (Dunn 2000, Smalley and Guries 1993). However, the impact of DED on wild elm populations in their native habitats is an equally important part of this story because any durable restoration requires that elms can co-evolve with the pathogen, which they can only do in the wild. Here, my aim is to show the impact that DED has had on native elm populations and their habitats in North America, focusing on American elm (*Ulmus americana* L.), the most widespread and common of the native North American elm species. To this end, I will present a synthesis of pertinent results from my own research in Connecticut River floodplain forests and published ecological research from across the native range of *U. americana*.

A review of a species' ecology is integral to its restoration for several reasons. First, given the many other threats affecting most ecosystems and the limited resources available for conservation, one needs a compelling rationale why restoring this particular species is a priority (Marks and Van Driesche 2016). For example, a key question to investigate is if natural selection has already increased the disease tolerance of the wild population to the level where demographic rates and elm forest structure are recovering unassisted by a breeding program. Second, when implementing a restoration, one should have a clear idea of what one is attempting to restore, including quantitative pre- and post-disturbance reference points against which to evaluate progress (e.g., Hanberry et al. 2012). Third, the leading cause of failure in plant reintroductions is that environmental conditions at reintroduction sites were inappropriate (Godefroid et al. 2011). This observation highlights the need for accurate quantitative measurements of species habitat requirements and preferences.

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Knowing well the ecology of American elm is all the more critical because it is a foundation species in river floodplains. A foundation tree species is a dominant canopy species whose architecture defines forest structure and whose traits control ecosystem dynamics and processes (Ellison et al. 2005). I will review the evidence that American elm was a codominant canopy species of floodplain forests of major rivers in the northeastern and prairie regions of North America before the spread of DED, as well as exploring the ecological role of American elm in those forests. Specifically, the review shows that the loss of American elm likely changed not just the composition of floodplain forests but also their structure, successional dynamics, and ecosystem processes. This finding lends support to the argument for investing in a disease-tolerance breeding and planting program to restore the wild population of American elm.

Distribution

The range of *U. americana* includes most of eastern North America (Bey 1990), but its relative abundance varies substantially across that range. Based on maps of the U.S. Forest Service's Forest Inventory and Analysis plot data, *U. americana* and the other native elm species have their greatest importance in the midwestern states in an area that roughly corresponds to the tallgrass prairie region, as well as in the Mississippi River alluvial plain, around Lake Erie and Lake Ontario, and into the Hudson River Valley (U.S. Forest Service, n.d.). Today, these regions are associated with intensive cultivation of corn, soybeans, and other agriculture (Hanberry et al. 2012). This association implies that like agriculture, elms are attracted to the most fertile soils. Maps of soil pH confirm that this region has soils with generally higher pH than the rest of eastern North America (U.S. Forest Service, n.d.). The distribution of highest *U. rubra* Muhl. abundance is even more strongly skewed toward this region of higher pH soils (U.S. Forest Service, n.d.). Up to the present, efforts to select disease tolerant elms from the native elm populations have been based mainly in northeastern North America where DED has occurred the longest. The observation that elm was historically also very abundant in the floodplain forests of the prairies and the Mississippi River alluvial plain suggests that exploration of these regions for exceptionally large surviving native elms may result in the discovery of additional disease tolerant selections (Whittemore and Olsen 2011).

Habitat and Succession

The Society of American Foresters lists *U. americana* as either a codominant or a commonly associated tree species in eight of its forest cover types (Table 1). What these forest types have in common is that they typically have seasonal flooding. Flooding acts not only as a periodic stress in creating hypoxic conditions in the soil, but also delivers sediments and nutrients with the floodwaters (Adair et al. 2004, Steiger and Gurnell 2003). Although all of these forest types occur on riverine floodplains, the black ash-American elm-red maple type can also occur in other types of swamps (Rudolf 1980).

A closer examination of Table 1 reveals that the elm-associated floodplain forest cover types actually represent different phases or regional variations on the same successional sequence (Table 2). This successional sequence starts with the formation of sand or gravel bar habitat in response to lateral river channel migration. Pioneer trees like *Salix* L. species and *Populus deltoides* W. Bartram ex Marshall are first to colonize the bars but are soon joined by *Acer saccharinum* L., which becomes dominant as the shorter-lived *Salix* and *P. deltoides* begin to die off (Table 2). Over time, sediments continue to accrete on the bar and annual flooding becomes less prolonged. On these older and better drained floodplain surfaces, the pioneer tree species are gradually replaced by *Fraxinus pennsylvanica* Marshall and *Ulmus americana*, two tree species that have both sufficient flood and shade tolerance to survive in the understory of the floodplain

Table 1.— Description of Society of American Foresters (SAF) forest cover types where American elm (*Ulmus americana*) is either a co-dominant species or a commonly associated species (Eyre 1980)

Number	Name	Description
39	Black ash – American elm – red maple	Most northern type of elm forest occurring well into the boreal region. It is found in swamps, gullies, and small depressions of slow drainage and along sluggish streams. Ash predominates on sites with higher pH whereas red maple predominates on more acidic sites, while American elm tends to grow on somewhat better drained sites.
61	River birch – sycamore	Floodplain forest type that may include American and slippery elms further back from the river. River birch is more dominant on streams flooded with acidic water because it is more tolerant of dissolved aluminum than other floodplain tree species.
62	Silver maple – American elm	Following cottonwood and willow in first bottoms of major rivers or pioneer community on abandoned floodplain fields. Relative proportion of maple to elm depends on history of stand.
63	Cottonwood	Characteristic of fronts and banks of most major streams. Cottonwood dominates the pioneer stage, but American elm can be an associated species in later stages.
65	Pin oak – sweetgum	A floodplain forest type with intermediate levels of flooding. American elm is one of many possible associated species.
93	Sugarberry – American elm – green ash	Major river floodplains at intermediate elevations in the floodplain. Appears to be long term in the successional scale because all type species are shade tolerant when small and reproduce readily.
94	Sycamore – sweetgum – American elm	River fronts in the first bottoms of major rivers, the banks of smaller rivers and large creeks that flood, and occasionally branch heads and coves of small creeks. Sites are rich with moderately good drainage and have adequate moisture throughout the growing season. Succeeds cottonwood on riverfront sites, but may be a pioneer forest on heavily cutover sites or old agricultural fields in floodplains. Where there are frequent flood disturbances, it may represent a persistent subclimax, but the climax will be swamp chestnut oak, cherrybark oak, or sweetgum – willow oak.
95	Black willow	Characteristic of fronts and banks of most major streams. Black willow is a temporary pioneer type. Cottonwood is the most common associate but American elm can be an associate in later stages. With succession, black willow is replaced by the silver maple – American elm type in the central region and by the sycamore – sweetgum – American elm type in the southern region.

Table 2.— Succession sequences of floodplain tree species on point bars and channel bars of major rivers. Sequences are inferred from transects across bar surfaces of increasing age (and elevation).

Successional sequence	Study location	Reference
Salix eriocephala – S. nigra – Populus deltoides – Acer saccharinum – Fraxinus pennsylvanica – Ulmus americana	Connecticut River in Massachusetts	Marks et al. 2014
Salix nigra – Acer negundo – A. saccharinum – Ulmus americana – Fraxinus nigra	Connecticut River in Vermont	Marks et al. 2014
Salix interior – S. nigra – Betula nigra – Fraxinus pennsylvanica – Ulmus americana – Celtis occidentalis	Chippewa River in Wisconsin	Barnes 1985, 1991
Salix amygdaloides – Populus deltoides – Acer negundo – Fraxinus pennsylvanica -Ulmus americana – Quercus macrocarpa	Missouri River in North Dakota	Johnson et al. 1976

forest (Table 2). The *F. pennsylvanica* - *U. americana* dominance will continue until sediments accrete to the point that the flood-intolerant tree species that dominate the surrounding upland forests can colonize (e.g., *Acer saccharum* Marshall*, Tilia americana* L.*, Quercus rubra* L., etc.) (Marks et al. 2014). Regional variations on this successional sequence are related to differences in the species pool associated with climate and soil pH. For example, on the drier western edge of its range, *U. americana* may be codominant with *Acer negundo* L. instead of *A. saccharinum* (Weaver et al. 1925, Wiebe and Shadick 2011). In riparian forests along medium and higher gradient streams and rivers, the pioneer tree species that *U. americana* eventually replaces can be *Platanus occidentalis* L. or on more acidic streams *Betula nigra* L. (Marks et al. 2014, McClelland and Ungar 1970, Oosting 1942). In particular, in the southern part of the *U. americana* range there are many more floodplain tree species such as *Celtis laevigata* Willd. and especially *Liquidambar styraciflua* L. that are associated with *U. americana* (Hanberry et al. 2012). Regardless of these regional variations, *U. americana* consistently appears to be most abundant in the better drained, older parts of forested point bars, riverine islands, and active floodplains where flooding is less frequent than in the *Salix*- and *P. deltoides* -dominated pioneer habitats but still frequent enough to prevent invasion by upland tree species (Marks et al. 2014). That transition zone where *U. americana* is most abundant occurs where flooding happens about 1 percent of the time (i.e., 4 days/year, on average) (Marks et al. 2014).

In the Connecticut River basin, the habitat of *U. rubra* is more restricted than that of *U. americana*. This fact may be underappreciated because even researchers have sometimes erroneously identified *U. americana* as *U. rubra*. Specifically, unlike *U. americana*, *U. rubra* appears to be generally restricted to higher floodplain terraces that do not flood every year (Curtis 1959, Marks et al. 2014). Moreover, within the Connecticut River watershed, *U. rubra* was further restricted to those high terraces that had the highest soil pH (Marks, unpublished data). The distribution of *Celtis occidentalis* L. was similarly restricted to sites with high soil pH, and is consequently a good indicator species for *U. rubra* habitat. The observation that *U. rubra* can be found on much drier sites outside riparian areas, particularly those of limestone origin (Cooley and Van Sambeek 1990), further emphasizes the importance of soil pH for this species.

A quantitative way to determine which tree species are most similar to native elm species ecologically is to review studies that did ordinations of floodplain forest species composition on multiple environmental gradients. Such ordinations show that *F. pennsylvanica* is ecologically the most consistently close to *U. americana,* and *C. occidentalis* the closest to *U. rubra* (Cowell and Dyer 2002, Meitzen 2009, Townsend 2001, Turner et al. 2004). The close ecological similarity of *F. pennsylvanica* implies that in many floodplain forests it has been able to replace *U. americana* in the canopy, thereby mitigating the impact of DED on these forests. Unfortunately, large numbers of mature *Fraxinus* trees are now also being lost across northeastern North America due to the invasive emerald ash borer (*Agrilus planipennis* Fairmaire 1888) (Flower et al. 2013, Knight et al. 2013). Although this pest will further impact late successional floodplain forests, the prospect of planting disease-tolerant selections of *U. americana* into large canopy gaps created by dead *Fraxinus* offers some hope for the ecological recovery of these forests (Knight et al. 2012).

It is interesting to observe that the European sister species of the North American elms have very similar habitat affinities (Richens 1983). *Ulmus laevis*, like *U. americana,* is primarily a species of floodplain forests where it replaces *Salix* and *Populus* pioneer species to become codominant with an ash species (*Fraxinus excelsior* L.) and *Quercus robur* L. (a sister species of *Q. bicolor* Willd.) in the later part of floodplain succession (Carbiener and Schnitzler 1990, Ellenberg 1988, Karpati and Toth 1961, Loiseau 1997, Margl 1973, Passarge 1956, Schnitzler 1995). *Ulmus glabra* Huds., like *U. rubra* flourishes on rich high floodplain terraces and in

ravines, but also occurs in some upland forests, principally on calcareous soils (Ellenberg 1988, Grime et al. 1988, Richens 1983, Schnitzler 1995). The *Ulmus*-related genera of *Planera* J. F. Gmel. and *Celtis* are likewise associated with floodplains and rich calcareous soils, respectively (Burns and Honkala 1990). This close ecological similarity implies a high level of niche conservatism in the family Ulmaceae. More importantly, the observation that all of these habitats have a high nutrient availability suggests that soil nutrient availability is of fundamental importance to the Ulmaceae.

Much prime elm habitat has been lost to clearing for agriculture because agriculture also prizes rich alluvial soils (Gerrard 1987). The construction of dams and levees has resulted in further losses of floodplain forest habitat (e.g., Johnson and Waller 2012, Johnson et al. 2012, Knutson and Klaas 1998). The increasing rarity of floodplain forests is making their protection and restoration a priority for both private and public conservation organizations (e.g., Hanberry et al. 2012, Nislow et al. 2010). The restoration of riparian buffers along streams in intensively cultivated agricultural fields is also a priority for the USDA Natural Resource Conservation Service programs. These parallel restoration efforts in prime elm habitat provide an opportunity for collaboration that could greatly augment capacity to implement plantings with disease tolerant selections of native elms across their range.

Dominance

The dominant plant species most strongly influence ecosystem processes like productivity, transpiration, and nutrient cycling. The tree species that have been abundant in a given region for a longer time also tend to have the most insect species associated with them (Southwood 1961). These plant species that dominate ecosystem structure and processes have been dubbed "foundation species" (Ellison et al. 2005). Given their large influence on ecosystem processes and structure, it has been argued that foundation species should be of greatest conservation concern when new threats, such as introduced pests and pathogens, emerge (Ellison et al. 2005).

Although never as common in eastern North America as dominant upland trees such as those in the genera *Quercus, Fagus* L., *Acer, Tsuga* Carrière, or *Pinus* L. (Thompson et al. 2013), *Ulmus* was frequently a dominant or codominant canopy tree species in its primary bottomland habitats before the spread of DED. For instance, in southern Ontario, *U. americana* was the leading dominant in forests of wet sites (Maycock 1963). In Connecticut River floodplain forests, *U. americana* was noted to be codominant with *A. saccharinum* (Nichols 1916). Even today, *U. americana* is second only to *A. saccharinum* in abundance in Connecticut River floodplain forests (Table 3). *U. americana* is also the most widespread, occurring in all floodplain forest types throughout the Connecticut River basin (Table 3). Floodplain forests in southern Quebec were likewise co-dominated by *A. saccharinum* and *U americana* before DED (Tessier et al. 1981). On the Upper Mississippi River, *U. americana* was codominant with *A. saccharinum* and *F. pennsylvanica*, and continues to be the second or third most abundant tree species (De Jager et al. 2012, Knutson and Klaas 1998). *U. americana* was dominant in some floodplain forests of the Wabash, Tippecanoe, and White Rivers in Indiana (Lee 1945, Lindsey et al. 1961), and continues to have very high densities in some Wabash River floodplains forests (Lindsey 2013). *U. americana* was also codominant in floodplain forests on major rivers in Wisconsin prior to the spread of DED, and continues to be one of the most common tree species in those floodplain forests (Curtis 1959, Hale et al. 2008, Johnson and Waller 2012, Turner et al. 2004). In floodplain forests of major rivers in Illinois, *U americana* was also codominant, especially in the more northern parts of the state and on the drier older parts of the floodplain (Hosner and Minckler 1963, Telford 1926, Thone 1922, Turner 1936). In the later stages of succession, *U. americana* was also codominant in floodplain forests in the western part of its

Table 3.— Composition of Connecticut River floodplain forests (Marks et al. 2014). Common species are listed in order of decreasing abundance. Uncommon species that were less than 0.5 percent of the trees are not included. Relative abundance measure used is frequency (i.e., percentage of all trees that belong to that species). Distribution refers to how widespread the tree species is measured as the percentage of (103) study sites where the species occurred in either the tree or in the shrub layer data. Species codes and nomenclature follows the USDA plants database (NRCS 2012).

Species scientific name	Species code	Relative abundance (% trees)	Distribution (% sites)
Acer saccharinum	ACSA2	23.93	71
Ulmus americana	ULAM	12.68	90
Acer rubrum	ACRU	8.48	60
Fraxinus pennsylvanica	FRPE	6.19	59
Acer saccharum	ACSA3	4.41	53
Acer negundo	ACNE2	4.27	49
Prunus serotina	PRSE2	3.77	64
Populus deltoides	PODE3	2.90	40
Carya cordiformis	CACO15	2.46	66
Platanus occidentalis	PLOC	2.27	35
Lindera benzoin	LIBE3	2.14	38
Carpinus caroliniana	CACA18	2.00	39
Quercus palustris	QUPA2	1.75	37
Pinus strobus	PIST	1.65	33
Fraxinus americana	FRAM2	1.59	50
Salix nigra	SANI	1.55	21
Alnus incana ssp. rugosa	ALINR	1.54	50
Quercus rubra	QURU	1.24	54
Tilia americana	TIAM	1.21	46
Viburnum lentago	VILE	0.97	36
Carya ovata	CAOV2	0.79	29
Tsuga canadensis	TSCA	0.68	17
Ilex verticillata	ILVE	0.66	34
Fagus grandifolia	FAGR	0.64	33
Ulmus rubra	ULRU	0.64	15
Betula lenta	BELE	0.62	19
Fraxinus nigra	FRNI	0.62	14
Rhus typhina	RHTY	0.57	17

range, including in Oklahoma (Bruner 1931, Collins et al. 1981, Hefley 1937, Little 1938, Rice 1965), Nebraska (Albertson and Weaver 1945, Weaver et al. 1925), North Dakota (Johnson et al. 1976), and Saskatchewan (Harms and Baker 1998, Wiebe and Shadick 2011). Even in the Lower Mississippi River Valley in southeastern Missouri where there are more competing species than further north, native elms were the most frequently recorded floodplain trees after *Liquidambar styraciflua* in General Land Office Surveys from the 19th century (Hanberry et al. 2012). Before DED arrived, *U americana* was sometimes also codominant in swamps in northeastern North America often with *Fraxinus nigra* Marshall and *Acer rubrum* L., (e.g., Barnes 1976, Meilleur et al. 1994). From this literature review we can conclude that before the spread of DED, *U. americana* was a codominant or even the most dominant canopy tree species in many floodplain forest stands across northeastern North America and westwards along the major rivers of the prairies.

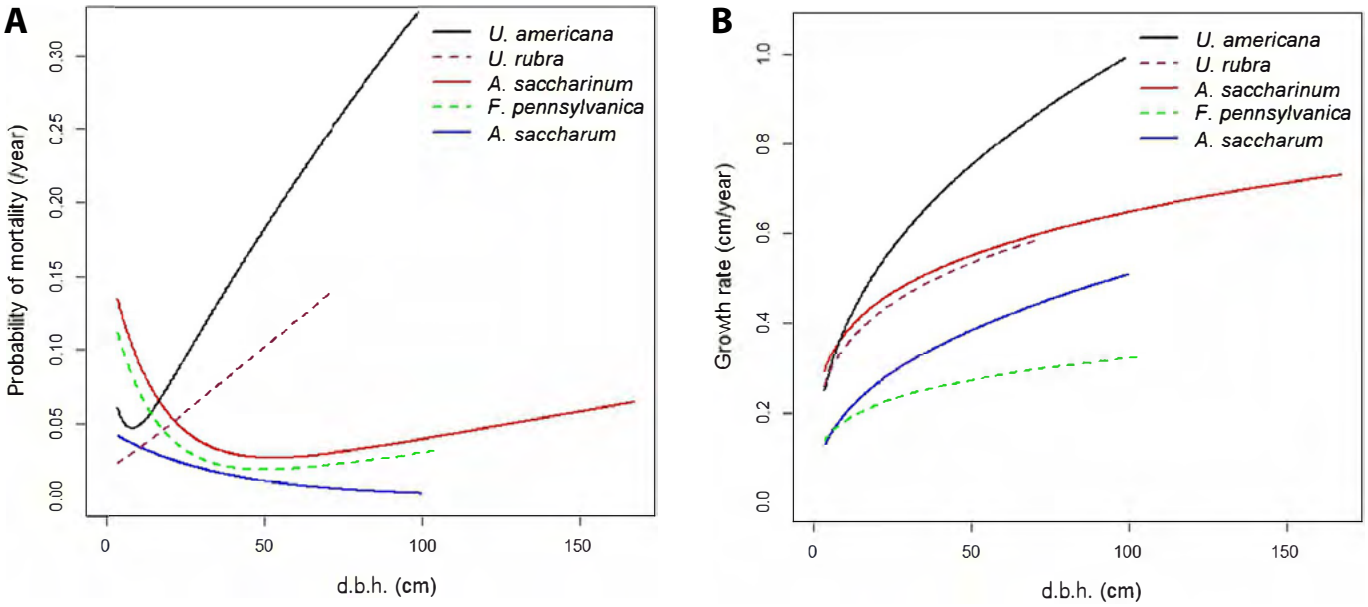

Figure 1.—Maximum likelihood estimates of size dependent average mortality (A) and diameter growth rates (B) for native elms and other common floodplain tree species of the Connecticut River Valley (Marks and Canham 2015). D.b.h. is the diameter at breast height. The study included more than 1,800 *U. americana* trees distributed across 90 floodplain forest sites from southern Connecticut to northern New Hampshire. Note that high growth rates are in part because floodplain species are fast growing, but also in part because floodplains have highly productive soils.

Demographic Decline

The first pandemic of Dutch elm disease in North America began with the arrival of the pathogen *Ophiostoma ulmi* (Buisman) Melin & Nannf. (1934) around 1927 (Brasier 2000). It was followed by a second pandemic caused by a more virulent strain of the pathogen *O. novo-ulmi*, likely starting in the 1940s (Brasier 2000). This second strain quickly became dominant and has now spread across almost the entire native range of American elm killing millions of trees (Brasier 2000). Unlike mature elms, elm seedlings are often spared. Seed production in American elm may begin as early as age 15 (Bey 1990). Around the same age, the probability of mortality due to DED begins to rise (Fig. 1A). Consequently, some of these small elms may produce another generation of seedlings before they are killed by DED, even if they do not have any significant tolerance to the disease. Therefore, although the average size of elm trees is expected to be much smaller after the arrival of DED, the number of elm trees may not decline as much. There is even the possibility of the number of elm trees increasing on some sites because each elm tree occupies less space. In other words, the initial demographic response to DED may differ depending on tree size class. Longer term, these population dynamics must result in a strong natural selection for earlier reproduction and/or increased ability to avoid and/or survive DED infection, potentially countering or even reversing a decline in the wild elm population depending on the amount of genetically-based variation available and its heritability. Thus data on the population trends in the wild population are highly pertinent to informing the prospects and necessity of disease tolerance breeding programs.

There is no systematic long-term monitoring program specifically designed for assessing elm population trends across the range of *U. americana*. However there have been several studies investigating the initial response of formerly elm-dominated forests to DED-induced elm mortality. The general pattern appears to be that there was a dramatic decline in total elm basal area in the affected stands as mature elms die from DED, but the response in the sapling and

small tree layer seems to be variable. Where elms were more dominant, larger canopy gaps resulted in more light, which allowed gaps to be invaded by shrubs (Dunn 1986, Huenneke 1983). Since that time, most of these shrub filled gaps have probably undergone succession back toward tree dominance, although there are no published followup studies. In other sites, canopy gaps created by elm mortality were filled by competing tree species even in the initial response, but there are also sites where sapling regeneration was mostly elms (Barnes 1976, Grittinger 1978, McBride 1973, Parker and Leopold 1983, Richardson and Cares 1976). Longer term studies of floodplain forests have also shown a dramatic decline in the number of large elms and total elm basal area, but a variable response in the seedling layer (Hale et al. 2008, Johnson and Waller 2012, Johnson et al. 2012, Knutson and Klaas 1998).

Demographic models of tree species allow investigating size dependent effects on mortality rates, which are clearly important in DED. In Connecticut River floodplain forests, where DED has occurred everywhere since the 1950s, both *U. americana* and *U. rubra* now have rapidly increasing mortality rates as a function of tree size (Fig. 1A). These mortality rates for mature elms are much higher than the mortality rates of similar sized trees of other species growing at the same sites (Fig.1A). Even though the high growth rate of elms can compensate somewhat for their high mortality rates (Fig. 1B), between the years 2008 and 2013 the population of modeled 30 cm (1 foot) d.b.h. elm trees in Connecticut River floodplain forests declined by 6.5 percent and 3.1 percent per year for *U. americana* and *U. rubra,* respectively (Marks and Canham 2015). The long-term rate of decline in the elm population may well be less severe than the measured rate from this relatively short study period because elm mortality may occur in waves (Brasier 1986). Likewise, it is possible that natural selection has increased the average disease tolerance in this elm population since the arrival of DED, but these high mortality rates especially for trees over 30 cm diameter at breast height (d.b.h.) (Fig. 1A) imply that the level of disease tolerance is still far from sufficient to allow native elms to reclaim their former role as codominant trees in the canopy of Connecticut River floodplain forests.

Forest Structure

"The well-known umbrella shape of the typical, planted, roadside elm is maintained to a large degree by the elm that grows in forest stands. The interlacing of the branches of adjacent trees occurs only near the top of the canopy, and conveys a strong resemblance to the arched vaulted ceiling of a cathedral" (Curtis 1959). Pictures of elm-dominated floodplain forests from before the arrival of DED are hard to find, but there are a few journal articles with black and white photos from the western part of the *U. americana* range (Albertson and Weaver 1945, Bruner 1931, Hefley 1937, Weaver 1960, Weaver et al. 1925). Here, I have included some color photos of elm-dominated floodplain forests on the Red River in eastern Saskatchewan, where DED did not arrive until after the year 2000 (Figs. 2-5). These color photos help convey the majesty of elm forests before the spread of DED.

The mounting risk of elm mortality due to DED with increasing tree size (Fig. 1A) has resulted in elms larger than 60 cm (2 feet) d.b.h. becoming rare in floodplain forests (Fig. 6). The few remaining elms that are larger usually occur in locations outside the floodplain where they are more than 90 m (300 feet) away from the nearest other remnant elms thereby reducing their chance of being visited by the bark beetles that spread DED. For instance, measurements in a Michigan elm forest in the wake of the initial wave of DED showed that elm mortality was highest in the lowland pockets where elm density was greatest (Richardson and Cares 1976). Due to this intense exposure to DED in floodplain forests where elms are most abundant, the size structure of the elm population in these forests has been dramatically reduced. Whereas in the 19th century elms were reported to be the largest trees in Massachusetts (Emerson 1887),

Figure 2.—Photo of the Rendek Elm Forest Sanctuary taken in the 1990s before the arrival of Dutch elm disease on the site around 2001. At the time the photo was taken, the canopy of this floodplain forest on the Red Deer River in eastern Saskatchewan was still dominated by *U. americana* (Harms and Baker 1998, Wiebe and Shadick 2011). Photo by Karen Wiebe, University of Saskatchewan, used with permission.

Figure 3.—Photo of the Rendek Elm Forest Sanctuary taken in the 1990s before the arrival of Dutch elm disease on the site around 2001. At the time the photo was taken, the canopy of this floodplain forest on the Red Deer River in eastern Saskatchewan was still dominated by *U. americana* (Harms and Baker 1998, Wiebe and Shadick 2011). The dominant fern in the forest understory is *Matteuccia struthiopteris*. Photo by Karen Wiebe, University of Saskatchewan, used with permission.

Figure 4.—Photo of floodplain forest dominated by *U. americana* on the south bank of the Red Deer River, Saskatchewan, about 800 m (0.5 miles) due west of the Manitoba border. The photo was taken on 24 July 1997, before Dutch elm disease arrived in this forest. The dominant fern in the forest understory is *Matteuccia struthiopteris*. Photo by Richard Kerbes, SOS Elms Coalition, Saskatoon, Saskatchewan, used with permission.

Figure 5.—Photo of floodplain forest dominated by *Ulmus americana* on the south bank of the Red Deer River, Saskatchewan, about 800 m (0.5 miles) due west of the Manitoba border. The photo was taken on 24 July 1997, before Dutch elm disease arrived in this forest. Photo by Richard Kerbes, SOS Elms Coalition, Saskatoon, Saskatchewan, used with permission.

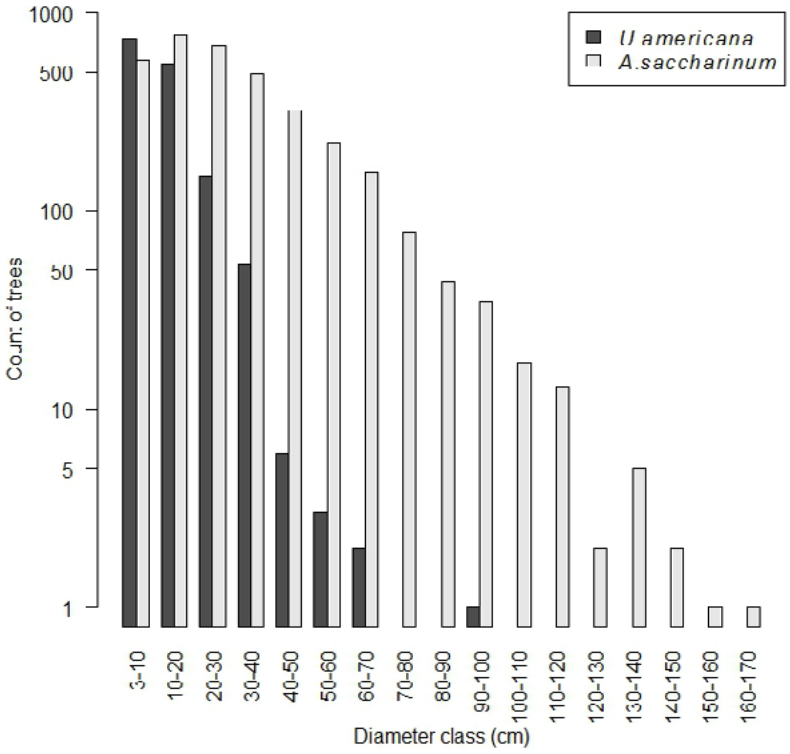

Figure 6.—Comparison of current tree size distributions for *U. americana* and *A. saccharinum*, the two most common floodplain tree species in the Connecticut River watershed. Size data were measured on the same sites for both species (Marks and Canham 2015). The one elm with a d.b.h. over 90 cm (3 feet) has died of DED since these data were collected.

today the silver maples (*A. saccharinum*) in Connecticut River floodplain forests are more than double the size of the elms (Fig. 6). Large reductions in the size distribution of floodplain forest elms have been measured across the elm range (Barnes 1997, Johnson and Waller 2012, Knutson and Klaas 1998, Richardson and Cares 1976). The 60 cm (2 feet) upper limit to the post-DED elm tree size distribution from the Connecticut River (Fig. 6) is remarkably consistent with these studies from other parts of the elm range. This observation suggests that exceptional native elms that have survived long enough to reach a size substantially larger than 60 cm (2 feet) d.b.h. despite the likely frequent past exposure to bark beetles in floodplain forest may well possess elevated tolerance to DED.

Given that the primary impact of DED on elm forests has been a dramatic reduction in the size of elms, it is a helpful point of reference for restoration to review the size and age that native elms can reach in the absence of DED. Specifically, a key question is if the rate of survival in a restored native wild elm population is sufficiently high that some elms will achieve a similar size and longevity as before the arrival of DED. My collaborators and I have been searching for the largest surviving elms in the Connecticut River, Housatonic River, and Lake Champlain valleys over the last 9 years to help identify native elms that may have elevated tolerance to Dutch elm disease. Based on our measurements of more than 250 exceptionally large elms scattered across this region, maximum d.b.h. are in the range of 92 to 184 cm (3 to 6 feet) and maximum heights are close to 33 m (110 feet) for both *Ulmus americana* and *U. rubra*. An inspection of state and national champion tree databases reveals that these maximum sizes are typical across eastern North America, although in exceptional cases *U. americana* can exceed 255 cm (8.5 feet) d.b.h. and 44 m (145 feet) in height, while the largest *U. rubra* recorded are over 225 cm (7.5 feet) d.b.h. and 37 m (125 feet) tall. Historic measurements of notable trees in Massachusetts and Connecticut from before the arrival of DED also record the largest individuals of *U. americana* at over 8 foot d.b.h. (Emerson 1887, Matthies 1934). The somewhat smaller maximum tree sizes among champion *U. rubra* compared with champion *U. americana* may be more of a consequence of there being far fewer *U. rubra* than *U. americana* trees (i.e., one is less likely to find an exceptional individual in a smaller sample). Whenever we have seen mature *U. rubra* and *U.*

americana growing together, trees of the two species were similar in height. Variation in height is much greater when comparing forest-grown and open-grown elms. Based on my measurements, forest-grown *U. americana* that live long enough to reach the canopy of floodplain forests can exceed 30 m (100 feet) in height while the range of heights for exceptionally large surviving open-grown *U. americana* is wider and less tall (65-90 feet or 20-27 m). Forest grown elms usually have a long main trunk before the first branching (Fig. 7), whereas open-grown elms often begin branching close to the ground to extend their crown out to the sides (Fig. 8). Likewise, there is geographic variation in tree size. We noticed that elms are shorter in the much colder northern part of the Connecticut River watershed than in the southern part of the watershed. There is probably more size variation across the broader range of *U. americana*. Although maximum diameters can be just as large in the western part of the *U. americana* range (e.g., Bronaugh 1993), elm canopy heights are notably less tall in the western-most part of the range and on drier sites (Albertson and Weaver 1945, Bey 1990).

U. americana was a long-lived species prior to the spread of DED, often reaching 175 to 200 years, with some trees older than 300 years (Bey 1990). Reports of elm ages for notable trees of Connecticut from before the arrival of DED are consistent with this range in longevity (Matthies 1934). I found a few measurements on ages of champion *U. americana* that also support this range in longevity. One measurement of a champion (6 foot or 185 cm d.b.h.) *U. rubra* in Vermont was around 150 years old when it died (exact age was not possible to determine because of decay) (Gus Goodwin, personal communication). Thus, exceptionally large elms may not be as old as they appear because elms can grow very quickly (Fig. 1B).

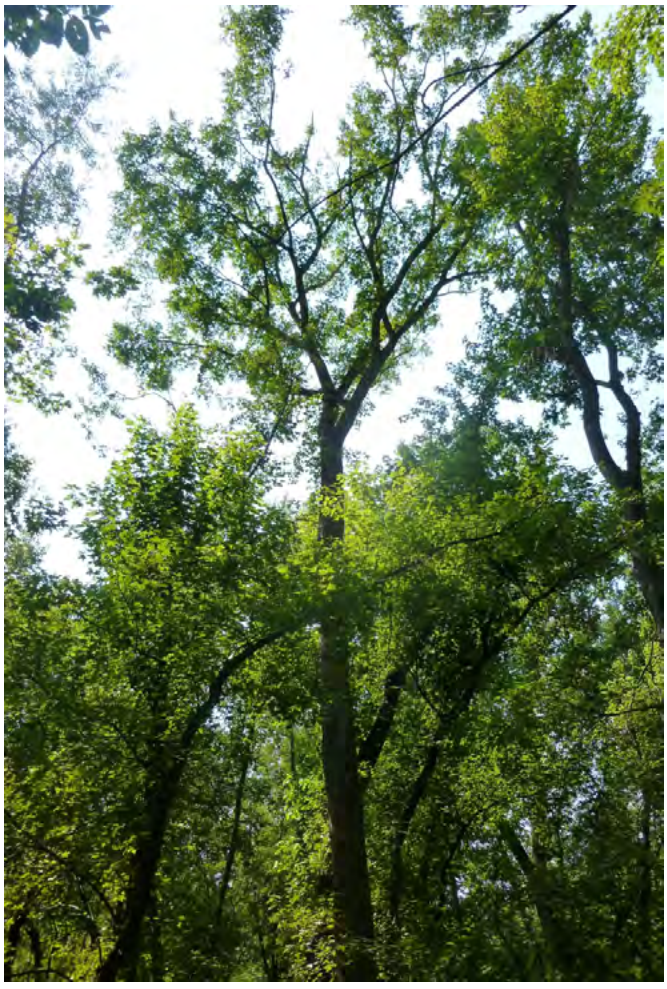

Figure 7.—Photo of an exceptionally large surviving American elm (*Ulmus americana*) in a Connecticut River floodplain forest in West Springfield, MA. This elm is 77 cm (>2.5 feet) d.b.h. and over 32.5 m (~110 feet) tall, and displays a straight trunk that is unbranched for at least the first 20 m (~65 feet), as is typical of forest-grown elms. The surrounding canopy trees are silver maple (*Acer saccharinum*). Photo by Christian O. Marks, The Nature Conservancy, used with permission.

Figure 8.—Photo of a lone, exceptionally large surviving American elm (*Ulmus americana*) on the bank of the Allagash River in northern Maine. This tree displays the tendency of many open-grown elms to start branching not far above the ground. Photo by Deborah Gardner, Mahoosuc Guide Service, used with permission.

Functional Traits

Elm species have unique traits that allow us to readily identify them in the field such as the alternating reddish-brown and cream-colored layers in the bark of *U. americana* (Wojtech 2011). Mature *Ulmus americana* and *U. laevis* also develop more pronounced buttress roots than other temperate trees, which helps identify them and adds to their aesthetic appeal (Richens 1983). Elm wood is also notoriously difficult to split because of its cross grain, a wood property that was sought after by wheelwrights (Richens 1983). Although these elm traits have utility for people and may well have some adaptive value to the trees in their native habitats, here I am concerned with traits that are functional in the sense that they affect ecosystem processes (*sensu* Garnier et al. 2016). Specifically, from a conservation perspective, a species loss is particularly consequential if that species possesses functional traits that differ substantially from the species that replace it. For example, the deciduous tree species that are replacing the evergreen conifer eastern hemlock (*Tsuga canadensis* (L.) Carrière) that have died due to invasive hemlock wholly adelgid have very different leaf traits than *Tsuga* and do not cast shade that is nearly as dark (Ellison et al. 2005).

Several primary axes of plant functional trait variation have been proposed (Westoby et al. 2002). Specifically, the leaf economics spectrum has been identified as a primary axis of functional trait variation globally (Wright et al. 2004). On this spectrum, leaf designs range from species with thick, tough, well-defended, long-lived leaves that have low metabolic rates and associated nitrogen content, to species with the opposite leaf attributes (Grime et al. 1988, Onoda et al. 2011, Reich et al. 1998b). These leaf traits have a strong influence on decomposition rates, and their palatability for herbivores, and consequently also on nutrient cycling (Cornelissen et al. 1999, Cornwell et al. 2008, Grime et al. 1988, Grime et al. 1996, Janzen 1974). Another important spectrum of trait variation is represented by the growth-survival tradeoff in trees, where fast-growing, short-lived species tend to have less dense wood with fewer defensive compounds than slow-growing, long-lived species (Wright et al. 2010). Wood density and tree longevity have obvious effects on forest carbon sequestration. Combining all of these traits, into a single global fast-slow economics spectrum for plants has been proposed (Lambers and Poorter

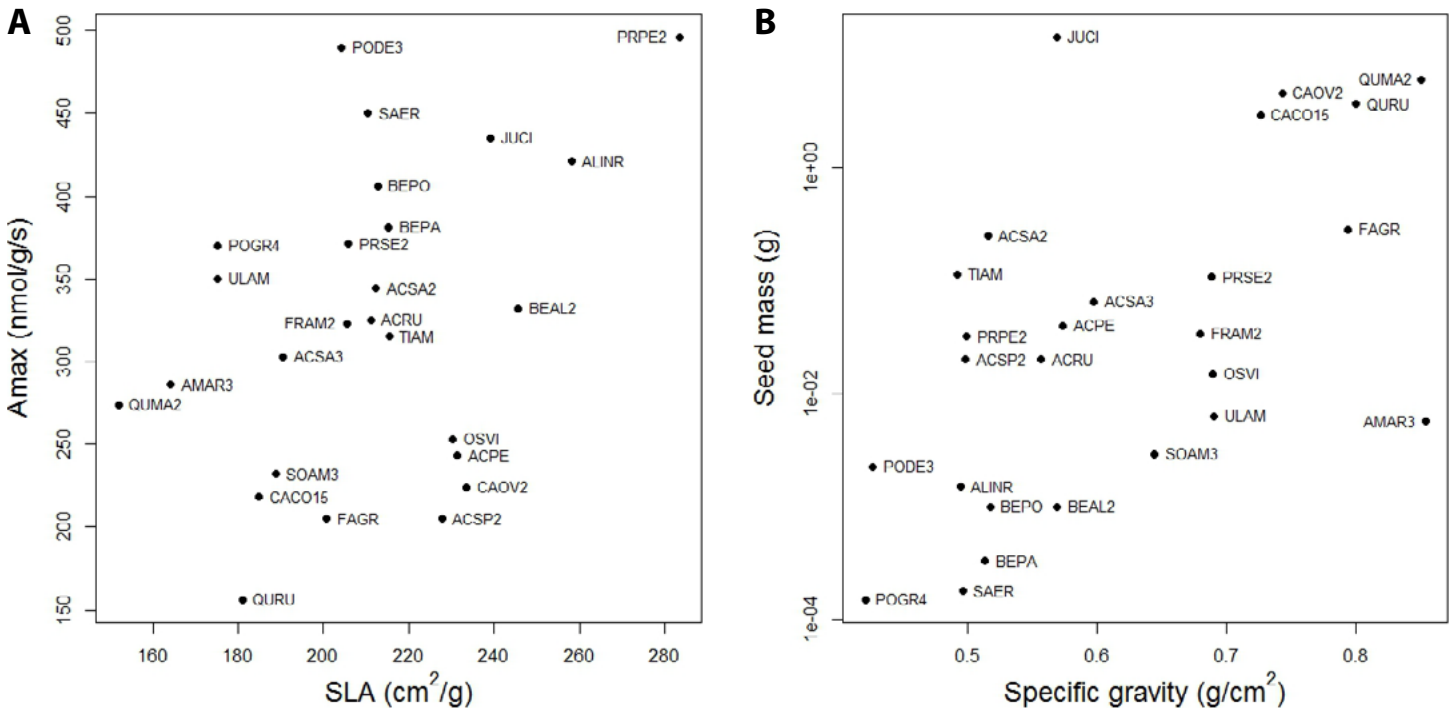

Figure 9.—Bivariate plots of functional trait values for 26 temperate deciduous forest tree and large shrub species from northeastern North America. (A) Data for maximum photosynthetic rate (Amax) and specific leaf area (SLA) are from Marks and Lechowicz (2007). There is a marginally significant positive relationship between Amax and SLA (p= 0.05806, adj. r^2= 0.1059); (B) Seedling wood specific gravity values are unpublished data from the experiment in Marks and Lechowicz (2007), and seed mass data were taken from the literature (Hewitt 1998, Young and Young 1992). There is a significant positive relationship between the log-transformed seed mass and wood specific gravity (p= 0.002827, adj. r^2= 0.287). The species acronyms in the plots are as follows: ACPE = *Acer pensylvanicum* L., ACRU = *Acer rubrum* L., ACSA2 = *Acer saccharinum* L., ACSA3 = *Acer saccharum* Marshall, ACSP2 = *Acer spicatum* Lam., ALINR = *Alnus incana* (L.) Moench ssp. *rugosa* (Du Roi) R.T. Clausen, AMAR3 = *Amelanchier arborea* (Michx. f.) Fernald, BEAL2 = *Betula alleghaniensis* Britton, BEPA = *Betula papyrifera* Marshall, BEPO = *Betula populifolia* Marshall, CACO15 = *Carya cordiformis* (Wangenh.) K. Koch, CAOV2 = *Carya ovata* (Mill.) K. Koch, FAGR = *Fagus grandifolia* Ehrh., FRAM2 = *Fraxinus americana* L., JUCI = *Juglans cinerea* L., OSVI = *Ostrya virginiana* (Mill.) K. Koch, PODE3 = *Populus deltoides* W. Bartram ex Marshall, POGR4 = *Populus grandidentata* Michx., PRPE2 = *Prunus pennsylvanica* L. f., PRSE2 = *Prunus serotina* Ehrh., QUMA2 = *Quercus macrocarpa* Michx., QURU = *Quercus rubra* L., SAER = *Salix eriocephala* Michx., SOAM3 = *Sorbus americana* Marshall, TIAM = *Tilia americana* L., ULAM = *Ulmus americana* L.. Species codes and nomenclature follows the USDA plants database (NRCS 2012).

2004, Reich 2014). A third important functional trait tradeoff is between producing many small seeds that can disperse to a wider surrounding area or producing fewer but larger seeds with more stored maternal reserves to support survival and establishment of the developing seedlings (Foster and Janson 1985, Leishman et al. 2000). Seeds are an important source of food for birds and mammals, with large "mast" seeds being especially valued by wildlife (Leishman et al. 2000). In forests, these plant functional trait spectra tend to follow succession from small-seeded, fast-growing pioneers to larger-seeded, slow-growing climax species (Reich et al. 1998a, 1998c).

A comparison of 26 temperate deciduous tree species from northeastern North America shows that *U. americana* falls near the middle of the trait range on this functional trait spectrum (see ULAM in Fig. 9). Not surprisingly, *Fraxinus*, which is most like elm in terms of habitat preferences and successional status, is also very close to elm on the functional trait spectra (compare FRAM2 to ULAM in Fig. 9A and 9B). Other species that frequently co-occur with *U. americana,* such as *A. saccharinum* and *A. rubrum*, likewise have similar functional trait values (see ACSA2 and ACRU, respectively, in Fig. 9A and 9B). Therefore, we should not expect a large impact on ecological function where elm was replaced in the forest canopy by these ecologically and functionally similar species following the spread of Dutch elm disease.

It is important to note that leaf traits, seed size, and wood specific gravity are not the only functionally important traits of trees. Loss of elms as codominant canopy trees may have affected ecological functions in floodplain forests in more subtle ways. For instance, a relatively

unique trait of elms is that their seeds ripen in late spring, a time when there are few other seeds available to granivorous birds and mammals. Although *A. saccharinum* and *A. rubrum* also produce their seeds in late spring, *Fraxinus* and most other northeastern North American tree species produce their seeds later in the year (Young and Young 1992). Another potentially unique characteristic of elms is that their wood is reputed to have exceptional resistance to decay when in continuous contact with water (Richens 1983). Given that the wood of fallen riparian elms will frequently end up in the water and that logs provide ecologically important habitat structure in stream channels (Collins et al. 2012, Schenk et al. 2014), decay resistance of submerged wood could be a functionally significant trait. Unfortunately, I do not know of any studies comparing durability of elm logs with logs of other species in streams. Studies comparing decomposition rates of logs on the ground showed that under those circumstances elm logs decay relatively quickly but decay rates of submerged logs could be substantially slower (Vrška et al. 2015).

Food Web

Farmers have fed elm leaves to their livestock since prehistoric times because of the relatively high palatability of elm (Grime et al. 1988, Richens 1983). As is typical of palatable plants, elm leaves have a low carbon-to-nitrogen ratio (C:N), a high pH, and decompose quickly (Ellenberg 1988). In the Connecticut River floodplain, beavers cut trees in the following order of frequency: *Salix, Populus, Fraxinus, Ulmus, Acer* (Marks and Canham 2015). My observations of vole girdling of tree seedlings planted in floodplains suggest that this rank order of genus preferences extends to rodent species other than beavers. In North America, there are more than 500 species of insects that are thought to be intimately associated with elm by either breeding, feeding, ovipositing, or hibernating in elms (Hoffmann 1942). Comparisons of tree genera for the number of insect species that feed on them show that *Ulmus* is ranked near the median both in eastern North America (Tallamy and Shropshire 2009) and Europe (Southwood 1961). Although counterintuitive, palatable plants like elm are actually expected to have fewer herbivorous insect species feeding on them because production of defensive compounds that reduce palatability evolve in response to more intensive insect feeding (Wratten et al. 1981). Nevertheless, native plants have many more caterpillars and other insects feeding on them than nonnative species (Burghardt et al. 2010, Southwood 1961). Thus the loss of large numbers of native elms and their frequent replacement by nonnative trees in cities may have had a significant impact on abundance of butterflies and moths as well as the success of nesting birds that prey on caterpillars (Burghardt et al. 2009).

Perhaps more important than the number of herbivores supported by elm is the number of insects that are specialized to elms as their primary host, because these species would be most threatened by a loss of elms (Table 4). In the case of the double-toothed prominent caterpillar

Table 4.—Preliminary list of caterpillars native to eastern North America that are specialized on *Ulmus* as their primary or even their exclusive host plant (Wagner 2010)

Common name	Species name	Type of caterpillar
Ochre dagger moth	*Acronicta morula*	Moth
Ruddy dagger moth	*Acronicta rubricoma*	Moth
Delightful dagger moth	*Acronicta vinnula*	Moth
Four-horned sphinx	*Ceratomia amyntor*	Moth
Double-lined prominent	*Lochmaeus bilineata*	Moth
Double-toothed prominent	*Nerice bidentata*	Moth
Question mark	*Polygonia interrogationis*	Butterfly

(*Nerice bidentata* Walker) this specialization appears to go beyond diet; the characteristic double-toothed dorsal keel of this caterpillar mimics the edge of an elm leaf (Wagner 2010). Insect species specialized to elms as their host have so far not been reported as threatened due to DED. This finding is not surprising, given that elm trees are still frequent in floodplain forests even if the total basal area of *Ulmus* trees has declined precipitously. However, these elm-specialist species could become threatened in the future should native elms continue to decline.

Ulmus americana and *U. rubra* are the first trees to flower in the spring, closely followed by *Acer rubrum* and *A. saccharinum* (Heinrich 1976, Marks unpublished data). The elms flower before leaf out when there is often still snow on the ground in New England. Although elms are primarily wind pollinated, their flowers are sometimes visited by insects, especially bees (Richens 1983). Likewise, herbivorous insects and their avian predators are attracted to the developing seeds of these tree species in early spring when most other trees are still bare. The early greening of floodplain trees may be one of the reasons that songbirds follow major rivers on their spring migration (Gauthreaux and Belser 2003, Kirsch et al. 2013).

Unlike most upland tree species whose seeds ripen in the summer or fall, the seeds of many floodplain tree species ripen in the spring to time their dispersal to coincide with the receding waters of the spring freshet. The order of floodplain tree seed dispersal roughly corresponds to the elevation of their preferred habitat. Specifically, *U. americana* seeds ripen first because they prefer the most well-drained parts of the active floodplain. They are followed by *A. rubrum* and *A. saccharinum*. Last of the spring seed producing trees are *P. deltoides* and *Salix* species because they are specialized to colonizing the most flood prone surfaces on new bars (Marks, unpublished data). Both avian and small mammal granivores consume elm seeds (Dulamsuren et al. 2009; Erritzoe 2010; Hulme and Hunt 1999; Perea et al. 2013, 2014; Venturas et al. 2014). Given that winter caches of seeds may be depleted by spring and few other plants produce seed at that time of year, elm seed could be locally important to granivore populations.

Conclusions and Perspectives

The greatest impact of DED on American elm has been on the population of canopy trees (i.e., trees >30 cm or >1 foot d.b.h). Unlike smaller elm trees, the number of elms that live long enough to reach the floodplain forest canopy have become rare (Fig. 6) because elm mortality rates increase dramatically with tree size (Figure 1A). One cannot talk about a recovery in the floodplain elm population until the *U. americana* mortality rate for canopy tree elms has come down to the range of mortality rates for other tree species (i.e., 3 to 5 percent/year). In controlled tests, currently the most disease-tolerant selections of *U. americana* can have a mortality rate as low as 4 percent after infection with the DED pathogen (e.g., Beier et al. 2017, this proceedings; Flower el al. 2017, this proceedings; Townsend and Douglass 2001), which implies that reaching the goal of an eventual recovery of the elm canopy tree population is plausible.

Ulmus americana fulfills the primary criterion of foundation species by having been a leading dominant of many forests, in this case floodplain forests of northeastern North America, the Mississippi Alluvial Valley, and along the rivers of the prairies. However, the loss of *U. americana* as canopy trees probably has not had as large of an effect on ecosystem processes and higher trophic levels as the loss of some other foundation species because the tree species that have replaced elms are ecologically and functionally very similar to elm. In particular, *A. saccharinum, A. rubrum*, and *Fraxinus* species, especially *F. pennsylvanica,* have similar functional traits and habitat preferences. Such ecological redundancy of tree species increases the resiliency of forests to disturbances, in this case, recovery of ecosystem processes and forest structure following the loss of canopy elms due to DED. However, with the invasive emerald ash borer now spreading

across the region and killing millions of *Fraxinus* trees, any "ecological insurance" from species redundancy has been used up in the region's floodplain forests. The reintroduction of canopy elms by planting disease tolerant *U. americana* selections in floodplain forests across the region to replace the *F. pennsylvanica* that have died would be a timely conservation action to help these increasingly rare communities recover some ecological resiliency (e.g., Knight et al. 2017, this proceedings).

The preferred habitat of elms are rich soils, in the case of *U. americana*, typically alluvium in the better drained parts of active floodplains (i.e., in the transition from dominance by floodplain pioneer tree species like *P. deltoides* and *A. saccharinum*, to upland tree species like *A. saccharum* and *Tilia americana,* which occurs where flooding happens around 1 percent of the time). Forests on relatively flat ground with rich soils such as many floodplain forests have been extensively cleared for agriculture. With a growing concern over stream water quality impacts of intensive agriculture, there are conservation programs that plant riparian buffers on streams passing through crop fields and pastures. This restoration of riparian buffers provides an opportunity to reintroduce native elms to these prime elm habitats by planting disease tolerant selections of *U. americana* (e.g., Knight et al. 2017, this proceedings).

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Literature Cited

Adair, E.C.; Binkley, D.; Andersen, D.C. 2004. **Patterns of nitrogen accumulation and cycling in riparian floodplain ecosystems along the Green and Yampa rivers.** Oecologia. 139: 108-116.

Albertson, F.W.; Weaver, J.E. 1945. **Injury and death or recovery of trees in prairie climate.** Ecological Monographs. 15: 393-433.

Barnes, B.V. 1976. **Succession in deciduous swamp communities of southeastern Michigan formerly dominated by American elm.** Canadian Journal of Botany. 54: 19-24.

Barnes, W.J. 1985. **Population-dynamics of woody-plants on a river island.** Canadian Journal of Botany. 63: 647-655.

Barnes, W.J. 1991. **Tree populations on the islands of the Lower Chippewa River in Wisconsin.** Bulletin of the Torrey Botanical Club. 118: 424-431.

Barnes, W.J. 1997. **Vegetation dynamics on the floodplain of the Lower Chippewa River in Wisconsin.** Journal of the Torrey Botanical Society. 124: 189-197.

Beier, G.L.; Held, B.W.; Giblin, C.P.; Blanchette, R.A. 2017. **Effects of inoculation timing on symptom development in *Ulmus americana* L.** In: Pinchot, C.C.; Knight, K.S.; Haugen, L.M.; Flower, C.E.; Slavicek, J.M., eds. Proceedings of the American elm restoration workshop 2016; 2016 October 25-27; Lewis Center, OH. Gen. Tech. Rep. NRS-P-174. Newtown Square, PA: U.S. Department of Agriculture, Forest Service Northern Research Station: 30-36.

Bey, C.F. 1990. ***Ulmus americana* L. American elm.** In: Burns R.M.; Honkala B.H., tech. coords. 1990. Silvics of North America. Vol 2. Hardwoods. Agric. Handb. 654. Washington, DC: Forest Service, United States Department of Agriculture: 801-807.

Brasier, C.M. 1986. **The population biology of Dutch elm disease: its principle features and some implications for other host-pathogen systems.** Advances in Plant Pathology. 5: 53-118.

Brasier, C.M. 2000. **Intercontinental spread and continuing evolution of the Dutch elm disease pathogens.** In: Dunn C.P., ed. The elms: breeding, conservation, and disease management. Boston, MA: Kluwer: 61-72.

Bronaugh, W. 1993. **The biggest American elm.** American Forests. 38: 13-15.

Bruner, W.E. 1931. **The vegetation of Oklahoma.** Ecological Monographs. 1: 99-188.

Burghardt, K.T.; Tallamy, D.W.; Shriver, W.G. 2009. **Impact of native plants on bird and butterfly biodiversity in suburban landscapes.** Conservation Biology. 23: 219-224.

Burghardt, K.T.; Tallamy, D.W.; Philips, C.; Shropshire, K.J. 2010. **Non-native plants reduce abundance, richness, and host specialization in lepidopteran communities.** Ecosphere. https://doi.org/10.1890/ES10-00032.1.

Burns, R.M.; Honkala, B.H., tech. coords. 1990. **Silvics of North America. Vol. 2. Hardwoods.** Agric. Handb. 654. Washington, DC: United States Department of Agriculture, Forest Service. 877 p.

Campanella, T.J. 2011. **Republic of shade: New England and the American elm.** New Haven, Connecticut, USA: Yale University Press. 240 p.

Carbiener, R.; Schnitzler, A. 1990. **Evolution of major pattern models and processes of alluvial forest of the Rhine in the rift-valley (France Germany).** Vegetatio. 88: 115-129.

Collins, B.D.; Montgomery, D.R.; Fetherston, K.L.; Abbe, T.B. 2012. **The floodplain large-wood cycle hypothesis: A mechanism for the physical and biotic structuring of temperate forested alluvial valleys in the North Pacific coastal ecoregion.** Geomorphology. 139: 460-470.

Collins, S.L.; Risser, P.G.; Rice, E.L. 1981. **Ordination and classification of mature bottomland forests in north central Oklahoma.** Bulletin of the Torrey Botanical Club. 108: 152-165.

Cooley, J.H.; Van Sambeek, J.W. 1990. ***Ulmus rubra* Muhl. Slippery elm.** In: Burns R.M.; Honkala B.H., tech. coords. 1990. Silvics of North America. Vol. 2. Hardwoods. Agric. Handb. 654. Washington, DC: Forest Service, United States Department of Agriculture: 812-816.

Cornelissen, J.H.C.; Pérez-Harguindeguy, N.; Díaz, S.; Grime, J.P.; Marzano, B. [et al.]. 1999. **Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents.** New Phytologist. 143: 191-200.

Cornwell, W.K.; Cornelissen, J.H.C.; Amatangelo, K.; Dorrepaal, E.; Eviner, V.T. [et al.]. 2008. **Plant species traits are the predominant control on litter decomposition rates within biomes worldwide.** Ecology Letters. 11: 1065-1071.

Cowell, C.M.; Dyer, J.M. 2002. **Vegetation development in a modified riparian environment: human imprints on an Allegheny River wilderness.** Annals of the Association of American Geographers. 92: 189-202.

Curtis, J.T. 1959. **The vegetation of Wisconsin: an ordination of plant communities.** Madison, WI: University of Wisconsin Press. 657 p.

De Jager, N.R.; Thomsen, M.; Yin, Y. 2012. **Threshold effects of flood duration on the vegetation and soils of the Upper Mississippi River floodplain, USA.** Forest Ecology and Management. 270: 135-146.

Dulamsuren, C.; Hauck, M.; Nyambayar, S.; Osokhjargal, D.; Leuschner, C. 2009. **Establishment of *Ulmus pumila* seedlings on steppe slopes of the northern Mongolian mountain taiga.** Acta Oecologica. 35: 563-572.

Dunn, C.P. 1986. **Shrub layer response to death of *Ulmus americana* in southeastern Wisconsin lowland forests.** Bulletin of the Torrey Botanical Club. 113: 142-148.

Dunn, C.P. 2000. **The elms: Breeding, conservation, and disease management.** Boston, MA: Kluwer. 361 p.

Ellenberg, H. 1988. **Vegetation ecology of central Europe.** Cambridge, UK: Cambridge University Press. 756 p.

Ellison, A.M.; Bank, M.S.; Clinton, B.D.; Colburn, E.A.; Elliott, K. [et al.]. 2005. **Loss of foundation species: consequences for the structure and dynamics of forested ecosystems.** Frontiers in Ecology and the Environment. 3: 479-486.

Emerson, G.B. 1887. **A report on the trees and shrubs growing naturally in the forests of Massachusetts.** Boston, MA: Little, Brown, and Co.

Erritzoe, Johannes. 2010. **Tree sparrows foraging on elm seeds for more than a month.** International Studies on Sparrows. 34: 23-24.

Eyre, F.H. 1980. **Forest cover types of the United States and Canada.** Bethesda, MD: Society of American Foresters.

Flower, C.E.; Knight, K.S.; Gonzalez-Meler, M.A. 2013. **Impacts of the emerald ash borer (*Agrilus planipennis* Fairmaire) induced ash (*Fraxinus* spp.) mortality on forest carbon cycling and successional dynamics in the eastern United States.** Biological Invasions. 15: 931-944.

Flower, C.E.; Pinchot, C.C.; Slavicek, J.M. 2017. **Restoring ecosystem resilience to urban forests using Dutch elm disease-tolerant American elm trees. In:** Pinchot, C.C.; Knight, K.S.; Haugen, L.M.; Flower, C.E.; Slavicek, J.M., eds. Proceedings of the American elm restoration workshop 2016; 2016 October 25-27; Lewis Center, OH. Gen. Tech. Rep. NRS-174. Newtown Square, PA: U.S. Department of Agriculture, Forest Service Northern Research Station: 141-142.

Foster, S.; Janson, C.H. 1985. **The relationship between seed size and establishment conditions in tropical woody plants.** Ecology. 66: 773-780.

Garnier, E.; Navas, M.-L.; Grigulis, K. 2016. **Plant functional diversity: organism traits, community structure, and ecosystem properties.** Oxford, UK: Oxford University Press. 256 p.

Gauthreaux, S.A., Jr.; Belser, C.G. 2003. **Radar ornithology and biological conservation.** The Auk. 120: 266-277.

Gerrard, J., ed. 1987. **Alluvial soils, Van Nostrand Reinhold Science Series.** New York, NY: Van Nostrand Reinhold. 305 p.

Godefroid, S.; Piazza, C.; Rossi, G.; Buord, S.; Stevens, A.-D. [et al.]. 2011. **How successful are plant species reintroductions?** Biological Conservation. 144: 672-682.

Grime, J.P.; Cornelissen, J.H.C.; Thompson, K.; Hodgson, J.G. 1996. **Evidence of a causal connection between anti-herbivore defence and the decomposition rate of leaves.** Oikos. 77: 489-494.

Grime, J.P.; Hodgson, J.G.; Hunt, R. 1988. **Comparative plant ecology: a functional approach to common British species.** London, UK: Unwin Hyman. 752 p.

Grittinger, T.F. 1978. **Loss of elm from some lowland forests in eastern Wisconsin.** Transactions of the Wisconsin Academy of Sciences and Arts Letters. 66: 195-205.

Hale, B.W.; Alsum, E.M.; Adams, M.S. 2008. Changes in the floodplain forest vegetation of the Lower Wisconsin River over the last fifty years. American Midland Naturalist. 160: 454-476.

Hanberry, B.B.; Kabrick, J.M.; He, H.S.; Palik, B.J. 2012. **Historical trajectories and restoration strategies for the Mississippi River Alluvial Valley.** Forest Ecology and Management. 280: 103-111.

Harms, V.; Baker, L. 1998. **A preliminary floral list for the Rendek Elm Forest Nature Sanctuary in east-central Saskatchewan.** Blue Jay. 56: 213-215.

Hefley, H.M. 1937. **Ecological studies on the Canadian River floodplain in Cleveland County, Oklahoma.** Ecological Monographs. 7: 345-402.

Heinrich, B. 1976. **Flowering phenologies: bog, woodland, and disturbed habitats.** Ecology. 57: 890-899.

Hewitt, N. 1998. **Seed size and shade-tolerance: a comparative analysis of North American temperate trees.** Oecologia. 114: 432-440.

Hoffmann, C.H. 1942. **Annotated list of elm insects in the United States.** Misc. Publ. 466. Washington, DC: U.S. Department of Agriculture. 20 p.

Hosner, J.F.; Minckler, L.S. 1963. **Bottomland hardwood forests of southern Illinois—regeneration and succession.** Ecology. 44: 29-41.

Huenneke, L.F. 1983. **Understory response to gaps caused by the death of *Ulmus americana* in central New York.** Bulletin of the Torrey Botanical Club. 110: 170-175.

Hulme, P.E.; Hunt, M.K. 1999. **Rodent post-dispersal seed predation in deciduous woodland: predator response to absolute and relative abundance of prey.** Journal of Animal Ecology. 68: 417-428.

Janzen, D.H. 1974. **Tropical blackwater rivers, animals, and mast fruiting by the Dipterocarpaceae.** Biotropica. 6(2): 69-103.

Johnson, W.C.; Burgess, R.L.; Keammerer, W.R. 1976. **Forest overstory vegetation and environment on Missouri River floodplain in North Dakota.** Ecological Monographs. 46: 59-84.

Johnson, W.C.; Dixon, M.D.; Scott, M.L.; Rabbe, L.; Larson, G.; Volke, M.; Werner, B. 2012. **Forty years of vegetation change on the Missouri River floodplain.** Bioscience. 62: 123-135.

Johnson, S.E.; Waller, D.M. 2012. **Influence of dam regulation on 55-year canopy shifts in riparian forests.** Canadian Journal of Forest Research. 43: 159-170.

Karpati, I.; Toth, I. 1961. **Die auenwaldtypen ungarns.** Acta Agronomica Academiae Scientiarum Hungaricae. 11: 421-452.

Kirsch, E.M.; Heglund, P.J.; Gray, B.R.; McKann, P. 2013. **Songbird use of floodplain and upland forests along the Upper Mississippi River corridor during spring migration.** The Condor. 115: 115-130.

Knight, K.S.; Brown, J.P.; Long, R.P. 2013. **Factors affecting the survival of ash (*Fraxinus* spp.) trees infested by emerald ash borer (*Agrilus planipennis*).** Biological Invasions. 15: 371-383.

Knight, K.S.; Haugen, L.M.; Pinchot, C.C.; Schaberg, P.G.; Slavicek, J.M. 2017. **American elm (*Ulmus americana*) in restoration plantings: a review. In:** Pinchot, C.C.; Knight, K.S.; Haugen, L.M.; Flower, C.E.; Slavicek, J.M., eds. Proceedings of the American elm restoration workshop 2016; 2016 October 25-27; Lewis Center, OH. Gen. Tech. Rep. NRS-174. Newtown Square, PA: U.S. Department of Agriculture, Forest Service Northern Research Station: 133-140.

Knutson, M.G.; Klaas, E.E. 1998. **Floodplain forest loss and changes in forest community composition and structure in the Upper Mississippi River: a wildlife habitat at risk.** Natural Areas Journal. 18: 138-150.

Lambers, H.; Poorter, H. 2004. **Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences.** Advances in Ecological Research. 34: 283-362.

U.S. Forest Service. N.d. **Climate change atlas.** Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Staion. http://www.nrs.fs.fed.us/atlas (accessed December 5, 2016).

Lee, M.B. 1945. **An ecological study of the floodplain forest along the White River system of Indiana.** Butler University Botanical Studies. 7: 155-175.

Leishman, M.R.; Wright, I.J.; Moles, A.T.; Westoby, M. 2000. **The evolutionary ecology of seed size.** Seeds: the ecology of regeneration in plant communities. 2: 31-57.

Lindsey, A.A. 2013. **Analysis of an original forest of the lower Wabash floodplain and upland.** Proceedings of the Indiana Academy of Science. 72: 282-287.

Lindsey, A.A.; Sterling, D.K.; Petty, R.O.; Vanasdal,W. 1961. **Vegetation and environment along Wabash and Tippecanoe Rivers.** Ecological Monographs. 31: 105-156.

Little, E.L., Jr. 1938. **The vegetation of Muskogee County, Oklahoma.** American Midland Naturalist. 19(3): 559-572.

Loiseau, J. 1997. **Flore et végétation des alluvions de la Loire et de l'Allier en Nivernais-Berry.** Journal de Botanique de la Société Botanique de France. 2: 27-44.

Margl, H. 1973. **Pflanzengesellschaften und ihre standortgebundene verbreitung in teilweise abgedämmten Donauauen (Untere Lobau).** Verhandlungen der Zoologischen und Botanischen Gesellschaft Wien. 113: 5-52.

Marks, C.O.; Canham, C.D. 2015. **A quantitative framework for demographic trends in size-structured populations: analysis of threats to floodplain forests.** Ecosphere. 6(11): 232. https://doi.org/10.1890/ES15-00068.1.

Marks, C.O.; Lechowicz, M.J. 2007. **The ecological and functional correlates of nocturnal transpiration.** Tree Physiology. 27: 577-584.

Marks, C.O.; Nislow, K.H.; Magilligan, F.J. 2014. **Quantifying flooding regime in floodplain forests to guide river restoration.** Elementa Science of the Anthropocene. 2: 000031. https:doi.org/10.12952/journal.elementa.000031.

Marks, C.O.; Van Driesche, R.G. 2016. **Designing restoration programs based on understanding the drivers of ecological change.** In: Van Driesche R. [et al.], eds. Integrating biological control into conservation practice. New York, NY: John Wiley and Sons: 4-21.

Matthies, K. 1934. **Trees of note in Connecticut.** New Haven, CT: Yale University Press. 34 p.

Maycock, P.F. 1963. **The phytosociology of the deciduous forests of extreme southern Ontario.** Canadian Journal of Botany. 41: 379-438.

McBride, J. 1973. **Natural replacement of disease-killed elms.** American Midland Naturalist. 90(2): 300-306.

McClelland, M.K.; Ungar, I.A. 1970. **The influence of edaphic factors on *Betula nigra* L. distribution in southeastern Ohio.** Castanea. 35: 99-117.

Meilleur, A.; Bouchard, A.; Bergeron, Y. 1994. **The relation between geomorphology and forest community types of the Haut-Saint-Laurent, Quebec.** Vegetatio. 111: 173-192.

Meitzen, K.M. 2009. **Lateral channel migration effects on riparian forest structure and composition, Congaree River, South Carolina, USA.** Wetlands. 29: 465-475.

Natural Resources Conservation Service (NRCS). 2012. **The Plants database.** Greensboro, NC: U.S. Department of Agriculture, Natural Resources Conservation Service. http://plants.usda.gov. (accessed September 10, 2012).

Nichols, G.E. 1916. **The vegetation of Connecticut V: plant societies along rivers and streams.** Bulletin of the Torrey Botanical Club. 43: 235-264.

Nislow, K.H.; Marks, C.O.; Lutz, K.A. 2010. **Aquatic conservation planning at a landscape scale.** In: Trombulak S.C.; Baldwin R.F., eds. Landscape-scale conservation planning. Dordrecht, Netherlands: Springer: 99-119. Chapter 6.

Onoda, Y.; Westoby, M.; Adler, P.B.; Choong, A.M.F.; Clissold, F.J. [et al.]. 2011. **Global patterns of leaf mechanical properties.** Ecology Letters. 14: 301-312.

Oosting, H.J. 1942. **An ecological analysis of the plant communities of Piedmont, North Carolina.** American Midland Naturalist. 28(1): 1-126.

Parker, G.R.; Leopold, D.J. 1983. **Replacement of *Ulmus americana* L in a mature east-central Indiana woods.** Bulletin of the Torrey Botanical Club. 110: 482-488.

Passarge, H. 1956. **Vegetationskundliche untersuchungen in Wäldern und Gehölzen der Elbaue.** Archiv für Forstwesen. 5: 339-358.

Perea, R.; Venturas, M.; Gil, L. 2014. **Seed predation on the ground or in the tree? Size-related differences in behavior and ecology of granivorous birds.** Acta Ornithologica. 49(1): 119-130.

Perea R.; Venturas, M.; Gil, L. 2013. **Empty seeds are not always bad: simultaneous effect of seed emptiness and masting on animal seed predation.** PLoS One. 8(9). 9 p. https://doi.org/10.1371/journal.pone.0065573.

Reich, P.B. 2014. **The world-wide 'fast–slow' plant economics spectrum: a traits manifesto.** Journal of Ecology. 102: 275-301.

Reich, P.B.; Tjoelker, M.G.; Walters, M.B.; Vanderklein, D.W.; Buschena, C. 1998a. **Close association of RGR, leaf and root morphology, seed mass, and shade tolerance in seedlings of nine boreal tree species grown in high and low light.** Functional Ecology. 12: 327-338.

Reich, P.B.; Walters, M.B.; Ellsworth, D.S.; Vose, J.M.; Volin, J.C.; Gresham, C.; Bowman, W.D. 1998b. **Relationships of leaf dark respiration to leaf nitrogen, specific leaf area and leaf life-span: a test across biomes and functional groups.** Oecologia. 114: 471-482.

Reich P.B.; Walters, M.B.; Tjoelker, D.; Vanderklein, D.; Buschena, C. 1998c. **Photosynthesis and respiration rates depend on leaf and root morphology and nitrogen concentration in nine boreal tree species differing in relative growth rate.** Functional Ecology. 12: 395-405.

Rice, E.L. 1965. **Bottomland forests of north-central Oklahoma.** Ecology. 46: 708-714.

Richardson, C.J.; Cares, C.W. 1976. **An analysis of elm (*Ulmus americana*) mortality in a second-growth hardwood forest in southeastern Michigan.** Canadian Journal of Botany. 54: 1120-1125.

Richens, R.H. 1983. **Elm.** Cambridge, UK: Cambridge University Press.

Rudolf, P. 1980. **Black ash - American elm - red maple.** In: Eyre, F., ed. Forest cover types of the United States and Canada. Bethesda, MD: Society of American Foresters: 37-38.

Schenk, E.; McCargo, J.; Moulin, B.; Hupp, C.; Richter, J. 2014. **The influence of logjams on largemouth bass (*Micropterus salmoides*) concentrations on the lower Roanoke River, a large sand-bed river.** River Research and Applications. 31(6): 704-711.

Schnitzler, A. 1995. **Successional status of trees in gallery forest along the River Rhine.** Journal of Vegetation Science. 6: 479-486.

Smalley, E.B.; Guries, R.P. 1993. **Breeding elms for resistance to dutch elm disease.** Annual Review of Phytopathology. 31: 325-354.

Southwood, T.R.E. 1961. **The number of species of insect associated with various trees.** Journal of Animal Ecology. 30: 1-8.

Steiger, J.; Gurnell, A.M. 2003. **Spatial hydrogeomorphological influences on sediment and nutrient deposition in riparian zones: observations from the Garonne River, France.** Geomorphology. 49: 1-23.

Tallamy, D.W.; Shropshire, K.J. 2009. **Ranking lepidopteran use of native versus introduced plants.** Conservation Biology. 23: 941-947.

Telford, C.J. 1926. **Third report on a forest survey of Illinois.** Illinois Natural History Survey Bulletin. 16: 1-122.

Tessier, C.; Maire, A.; Aubin, A. 1981. **Study of the riparian vegetation in a group of 30 islands in the Saint-Lawrence River, Quebec.** Canadian Journal of Botany. 59(8): 1526-1536.

Thompson, J.R.; Carpenter, D.N.; Cogbill, C.V.; Foster, D.R. 2013. **Four centuries of change in northeastern United States forests.** PLoS One. 8(9): e72540. 15 p. https://doi.org/10.1371/journal.pone.0072540.

Thone, F. 1922. **Ecological factors in region of Starved Rock, Illinois.** Botanical Gazette. 345-368.

Townsend, A.; Douglass, L. 2001. **Variation among American elm clones in long-term dieback, growth, and survival following *Ophiostoma* inoculation.** Journal of Environmental Horticulture. 19: 100-103.

Townsend, P.A. 2001. **Relationships between vegetation patterns and hydroperiod on the Roanoke River floodplain, North Carolina.** Plant Ecology. 156: 43-58.

Turner, L.M. 1936. **Ecological studies in the Lower Illinois River Valley.** Botanical Gazette. 97(4): 689-727.

Turner, M.G.; Gergel, S.E.; Dixon, M.D.; Miller, J.R. 2004. **Distribution and abundance of trees in floodplain forests of the Wisconsin River: environmental influences at different scales.** Journal of Vegetation Science. 15: 729-738.

Venturas, M.; Nanos, N.; Gil, L. 2014. **The reproductive ecology of *Ulmus laevis* Pallas in a transformed habitat.** Forest Ecology and Management. 312: 170-178.

Vrška, T.; Přívětivý, T.; Janík, D.; Unar, P.; Šamonil, P.; Král, K. 2015. **Deadwood residence time in alluvial hardwood temperate forests: a key aspect of biodiversity conservation.** Forest Ecology and Management. 357: 33-41.

Wagner, D.L. 2010. **Caterpillars of eastern North America: a guide to identification and natural history.** Princeton, NJ: Princeton University Press. 512 p.

Weaver, J. 1960. **Flood plain vegetation of the central Missouri Valley and contacts of woodland with prairie.** Ecological Monographs. 30: 37-64.

Weaver, J.E.; Hanson, H.C.; Aikman, J.M. 1925. **Transect method of studying woodland vegetation along streams.** Botanical Gazette. 80: 168-187.

Westoby, M.; Falster, D.S.; Moles, A.T.; Vesk, P.A.; Wright, I.J. 2002. **Plant ecological strategies: some leading dimensions of variation between species.** Annual Review of Ecology and Systematics. 33: 125-159.

Whittemore, A.T.; Olsen, R.T. 2011. ***Ulmus americana* (Ulmaceae) is a polyploid complex.** American Journal of Botany. 98: 754-760.

Wiebe, K.L.; Shadick, S. 2011. **Birds associated with American elm forests in northeastern Saskatchewan.** Blue Jay. 69: 161-167.

Wojtech, M. 2011. **Bark: a field guide to trees of the Northeast.** Lebanon, NH: University Press of New England. 280 p.

Wratten, S.D.; Goddard, P.; Edwards, P.J. 1981. **British trees and insects: the role of palatability.** The American Naturalist. 118: 916-919.

Wright, I.J.; Reich, P.B.; Westoby, M.; Ackerly, D.D.; Baruch, Z. [et al.]. 2004. **The worldwide leaf economics spectrum.** Nature. 428: 821-827.

Wright, S.J.; Kitajima, K.; Kraft, N.J.B.; Reich, P.B.; Wright, I.J. [et al.]. 2010. **Functional traits and the growth–mortality trade-off in tropical trees.** Ecology. 91: 3664-3674.

Young, J.A.; Young, C.G. 1992. **Seeds of woody plants in North America.** Portland, OR: Timber Press. 416 p.

ELM GENETIC DIVERSITY AND HYBRIDIZATION IN THE PRESENCE OF DUTCH ELM DISEASE

Johanne Brunet and Raymond P. Guries[1]

Abstract.—The impact of Dutch elm disease (DED) on the genetic diversity of slippery elm (*Ulmus rubra)* is summarized and its potential impact on the genetic diversity of other North American native elms, American elm (*U. americana)*, rock elm (*U. thomasii)*, winged elm (*U. alata*), cedar elm (*U. crassifolia*), and September elm (*U. serotina),* is discussed. The potential for hybridization between the introduced Siberian elm *U. pumila* and the native North American elms is considered given previous findings with *U. rubra*. We do not expect DED to reduce the genetic diversity of these native elms. The only exception may be *U. serotina* if its restricted range leads to genetic discontinuities among populations. We do not expect hybridization between *U. americana* and *U. pumila* due to incompatibility barriers, but hybridization between *U. pumila* and other native elms appears more likely and could have negative effects on the long term conservation of these species. This information is timely given the current efforts to restore American elm across the U.S. landscape.

Introduction

Dutch elm disease (DED), caused by the introduced fungal pathogen *Ophiostoma ulmi*, has devastated North American elm populations for more than 75 years. The first wave of DED in North America began around 1930 (Smalley and Guries 1993) and quickly decimated both rural and urban populations. Currently, a new sub-species, *O. novo-ulmi*, even more virulent than the first, continues to ravage native elm populations (Brasier 2000). All native North American elm species, including the iconic American elm, *Ulmus americana*, have been affected by DED. Although elms have not been eliminated from forest settings (Richardson and Cares 1976), there was tremendous mortality due to DED, which resulted in a smaller percentage of large diameter native elm trees in eastern hardwood forests, based on reports after 20 years of DED pressure in an east-central Indiana forest (Parker and Leopold 1983). Although precise estimates of the numbers of elms lost to DED are unknown, the loss has been estimated into the hundreds of millions of trees based on their former abundance (Bey 1990).

In response to the disease, resistance to DED was examined in different elm species worldwide. Little resistance was detected in most native elm species of Europe and North America but it was identified in some Asian elm species, including the Siberian elm, *U. pumila*. Resistance to DED is variable in *U. pumila* and some accessions have been used in attempts to breed DED resistance into native North American elm species. In fact, *U. pumila* has served as the source of DED resistance in virtually every new Eurasian elm cultivar released in the United States since the 1960s (Smalley and Guries 1993). The development of DED-resistant hybrid elms led to the replacement of North American elms with Eurasian hybrids on urban boulevards, but forest losses to the disease continue due to the lack of DED resistance in North American elms (Smalley and Guries 1993).

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Breeding programs in the United States failed to introduce DED resistance from Eurasian species into *U. americana*. While many believed ploidy level differences accounted for the inability to cross *U. americana* with DED-resistant species, an incompatibility barrier may provide a more likely explanation (Ager and Guries 1982). *Ulmus americana* is a tetraploid (4n=56) while other elms are diploid (2n=28), although Whittemore and Olsen (2011) have recently reported that more than 20 percent of 81 *U. americana* trees from a range-wide collection were diploid. What progress has been made in developing putatively disease resistant *U. americana* trees has come from selections drawn from *U. americana* seedling populations. This finding suggests that development of DED-resistant *U. americana* may be possible without recourse to breeding with resistant Eurasian elms.

Dutch elm disease could have decreased the genetic diversity of native North American elm species. The demographic changes and reduction in population sizes associated with DED losses could create genetic bottlenecks and alter the level of genetic diversity within elm populations and also modify how such genetic diversity is distributed over the landscape (genetic differentiation) (Bouzat 2010). In addition, although *U. pumila* was introduced to the United States prior to the first DED pandemic (Ware 1995), it was largely unaffected by it due to its resistance to DED and its tolerance of dry climatic conditions (Ding et al. 2006, Leopold 1980). *Ulmus pumila* trees were planted to serve as wind breaks along highways, near farms, villages and towns, and in urban landscapes to replace dead or dying DED-susceptible native elms (Ware 1995). *Ulmus pumila* has naturalized (Zalapa et al. 2009) and now occurs throughout the range of native U.S. elms and has been declared invasive in some states (Kartesz 2015, NRCS 2017). Given the ability of *U. pumila* to interbreed with some North American elms (e.g., slippery elm or *U. rubra*; Zalapa et al. 2009, 2010), we expect the risk of hybridizing with native elm species to increase over time.

We have previously examined the impact of DED on the genetic diversity of one native North American elm species, *U. rubra*, common in Wisconsin and much of the eastern United States (Brunet et al. 2016). We have also examined the levels of hybridization between the native *U. rubra* and invasive *U. pumila* in Wisconsin (Zalapa et al. 2009, 2010). In the current study, we summarize these results and discuss how we expect DED and the spread of *U. pumila* across the U.S. landscape to influence other native elm species, besides *U. rubra*. We address the potential impact of DED on the genetic diversity of these native elms and also discuss the potential for hybridization between other native elm species and the introduced *U. pumila*. These questions are timely, given the current program to restore *U. americana* across the U.S. landscape. This study also illustrates how efforts to combat the negative impacts of a disease epidemic may have unforeseen consequences for native elm populations.

Materials and Methods

Plant Materials

Elm accessions used in this research were drawn from a variety of sources and represent both fresh and herbarium specimens (Table 1). Fresh specimens were collected relatively recently (2005-2007) from wild populations; collection of herbarium specimens took place between 1890 and 2004. Identification of species and their putative hybrids were made at the time of collection using a suite of morphological traits. The leaves, buds, twigs, and seed characteristics used for identification of each parental species, *U. rubra* or *U. pumila*, are described in Table 2 of Zalapa et al. (2010). The *U. pumila* accessions comprised samples from Asia, live trees collected throughout the United States, and live trees and herbarium specimens from Wisconsin (Table 1). Accessions of *U. rubra* originated mostly from Wisconsin and represented both live trees and herbarium specimens (Table 1).

Table 1.—Locations, sample sizes, and reference for *Ulmus pumila* accessions collected in the United States and East Asia and of *U. rubra* accessions collected in Wisconsin

Location	N	Reference
Ulmus pumila		
1. United States accessions		
Live trees from KY, KS, OR, CO, AZ, IA, OK, OH, PA, UT, TX, DE, GA, NJ, MN, AK, IL, IN, VA, WA, TN, SD, MO, MA, NV, LA, NY, MD.	37	Zalapa et al. 2010
2. Wisconsin accessions		
a) Live trees from 6 wild U. pumila populations with morphological hybrids.	95	Zalapa et al. 2009
b) Live trees from 8 wild populations from WI, SD, IL with no morphological hybrids	171	Zalapa et al. 2010
c) Wisconsin herbarium accessions collected throughout WI between 1948 and 2001	52	Zalapa et al. 2010
3. Accessions from East Asia		
72 China (15 Henan, 13 Shanxi, 10 Hebei, 7 Xinjiang, 6 Hubei, 5 Beijing, 5 Heilongjiang, 3 Ganzu, 3 Shandong, 2 Liaoning, 2 Guizhou, and 1 Shaanxi), 9 Russia, 1 Korea, and 4 Morton Arboretum.	86	Zalapa et al. 2010
Ulmus rubra		
Wisconsin accessions		
UW-Herbarium specimens, Madison, WI. Collected throughout WI between 1890 and 2004. Thirty-eight specimens were collected before 1960 (pre-DED) and 39 between 1961 and 2004 (post-DED)	77	Brunet et al. 2016
Leaves from 100 living trees with 20 trees sampled in each of five wild U. rubra populations in Wisconsin	100	Zalapa et al. 2009, Brunet et al. 2016

Genetic Analysis

Elm accessions were genotyped using 9 to 13 microsatellite loci, previously described in Zalapa et al. (2009, 2010) and Brunet et al. (2016). The impact of DED on the genetic diversity of *U. rubra* was examined by comparing the genetic diversity of herbarium specimens pre- and post-DED. We also examined and compared the levels of genetic diversity of herbarium specimens to that of wild *U. rubra* populations (Brunet et al. 2016). Genetic diversity was quantified by the number of alleles and the level of heterozygosity, both calculated using Gene AlEx 6 (Peakall and Smouse 2006). In addition, we looked for evidence of genetic differentiation among wild *U. rubra* populations. Here, we used analyses of molecular variance (AMOVA) (Excoffier et al. 1992) and calculated pairwise F_{ST} in Gene AlEx to determine how the genetic diversity was distributed within and among populations. We also used a Bayesian clustering method available in the program STRUCTURE (Pritchard et al. 2000) to identify the number of genetic clusters in the data and identify potential genetic discontinuities among groups or populations. Principal coordinate analyses (PCoA) illustrated the distribution of genetic diversity among specific groups (Brunet et al. 2016).

Three different methods were used to identify genetic hybrids in naturalized *U. pumila* populations. First, the nine loci with species-specific alleles permitted direct identification of the genetic hybrids (Zalapa et al. 2010). Second, Bayesian clustering analyses implemented in the program STRUCTURE separated the two pure parental species of *U. pumila* and *U. rubra* from the hybrids (Zalapa et al. 2009, 2010). Finally, principal coordinate analyses (PCoA) helped illustrate the pattern of introgression (Zalapa et al. 2009). Details on the genetic analyses and the specific software employed can be found in Zalapa et al. (2009, 2010) and Brunet et al. (2016).

Table 2.—Genetic diversity of *Ulmus rubra* accessions. These data represent a subsample of the data presented in Table 3 of Brunet et al. 2016. Populations 1-5 are wild populations. N is sample size; H_e is the expected level of heterozygosity.

Accession	N	Number of alleles	Alleles per locus (SE)	H_e (SE)/
Population 1	20	62	4.8 (0.7)	0.51 (0.08)
Population 2	20	62	4.8 (0.8)	0.53 (0.07)
Population 3	20	79	6.1 (1.0)	0.60 (0.08)
Population 4	20	73	5.6 (0.9)	0.52 (0.08)
Population 5	20	75	5.8 (1.0)	0.56 (0.08)
All populations combined	100	106	8.1 (1.4)	0.56 (0.08)
All herbarium specimens	77	108	8.3 (1.4)	0.57 (0.08)
Pre-DED	38	92	7.1 (1.2)	0.57 (0.08)
Post-DED	39	97	7.5 (1.2)	0.58 (0.08)

Results

We obtained no evidence that DED reduced the genetic diversity within *U. rubra*. First, levels of genetic diversity did not change between the groups of pre- and post-DED herbarium specimens (Table 2). Second, levels of genetic diversity were similar between wild and herbarium specimens (Table 2). Finally, the levels of genetic diversity remained high in wild *U. rubra* populations (Table 2). Moreover, we found no evidence of genetic differentiation among wild *U. rubra* populations. The AMOVA results indicated low levels of genetic differentiation, where 96 percent of the genetic variation was observed within relative to 4 percent among populations. In addition, overall FST was low at 0.04 and FST values ranged between 0.018 and 0.055 among pairs of populations. Finally, the STRUCTURE results indicated the presence of three genetic clusters (K=3) in the five wild *U. rubra* populations, with ample mixing of the clusters within each population (See Fig. 2 in Brunet et al. 2016).

Our results support widespread hybridization between *U. pumila* and *U. rubra* in Wisconsin (Zalapa et al., 2009, 2010) and an asymmetric pattern of introgression toward *U. pumila* (Fig. 2 in Zalapa et al. 2009). Such a pattern of introgression indicates most of the first-generation (F1) hybrids backcross to *U. pumila* rather than to *U. rubra*. We also observed greater genetic diversity and new combination of alleles in *U. pumila* populations containing hybrids (Zalapa et al. 2009, 2010). Hybrids were common not only in *U. pumila* populations where we had originally detected morphological hybrids (Zalapa et al. 2009) but also in populations where no hybrids were suspected based on morphological observations (Zalapa et al. 2010). Out of 92 trees sampled from naturalized *U. pumila* populations in Zalapa et al. (2009), 51 trees were determined to be hybrids, and of these, 35 were first-generation hybrids and 16 were backcrosses, 14 back to *U. pumila* and 2 back to *U. rubra* (Table 2 in Zalapa et al. 2009). The hybrids had more alleles and greater heterozygosity than the pure *U. pumila* individuals (hybrids: 7.22 alleles and He = 0.90 vs. *U. pumila*: 2.78 alleles and He = 0.26). Moreover, in the 171 naturalized *U. pumila* trees collected for the Zalapa et al. 2010 study, 44 were identified as genetic hybrids and of these, 30 individuals were identified as F1 hybrids, 7 as first-generation backcross to *U. pumila* (BC1Pu) and 7 as second-generation backcross to *U. pumil*a (BC2Pu). The presence of hybrids always increased the genetic diversity of *U. pumila* populations, both the number of alleles and the level of heterozygosity (Table 2 in Zalapa et al. 2009 and Table 4 in Zalapa et al. 2010).

Discussion

Dutch elm disease did not decrease the level of genetic diversity within *U. rubra* (see Brunet et al. 2016 for details). Both the number of alleles and the level of heterozygosity remained essentially unchanged in the herbarium specimen post-DED collection relative to pre-DED levels, and these levels were also similar to those in present day natural *U. rubra* populations. Moreover, there was little genetic differentiation among natural *U. rubra* populations such that each population harbored similar levels of genetic diversity. A search of the literature on other forest trees indicated that species subjected to a serious disease epidemic experienced little or no impact on levels of genetic diversity (reviewed in Brunet et al. 2016).

The lack of reduction in genetic diversity following the introduction of a serious disease may be due to the fact that these trees are wind-pollinated. Pollen can move long-distances in wind-pollinated trees reducing population differentiation and allowing each population to harbor most of the genetic diversity characteristic of the species (Burczyk et al. 2004, Loveless and Hamrick 1984, Slatkin 1987). Under such conditions, even if many elm populations were decimated by disease, even a single remaining population would maintain most of the genetic diversity characteristic of the species. In general, levels of genetic diversity tend to be high in wind-pollinated trees (Brunet et al. 2016). In addition, the high level of heterozygosity observed within populations suggests that all (or most) of the alleles could be recovered from fewer individuals than if trees were more inbred. The high level of heterozygosity also reflects the fact that little selfing or biparental inbreeding (mating between close relatives) occurs in these elm populations (Brunet et al. 2016, Glémin et al. 2006). Therefore, high gene flow and low levels of inbreeding have allowed for the maintenance of high levels of genetic diversity in *U. rubra* despite the loss of a large number of *U. rubra* trees to DED. Despite long-term exposure of *U. rubra* to DED, the species remains relatively unchanged in its genetic constitution.

Of the six native elm species present in the United States, *U. americana* has the largest historic range, followed closely by *U. rubra;* both occur in the eastern and midwestern regions of the United States (NRCS 2017). The range of rock elm (*U. thomasii*) is more limited, as it is found primarily in the north-central United States and it is less common than *U. americana* or *U. rubra* in their shared range. Winged elm (*U. alata*) and cedar elm (*U. crassifolia)* are found in the southern parts of the United States while the range of September elm (*U. serotina)* is very limited in several southern states (NRCS 2017). Because all North American elm species are wind-pollinated and pollen has the potential to move long distances, we expect little genetic differentiation among populations. For genetic differentiation to occur, populations should be isolated by large geographic distances that limit gene flow, be small in size, or be exposed to different selection regimes (Slatkin 1987). The species where substantial genetic differentiation may be present is *U. serotina*, given its restricted range. Future studies should assess the level of genetic differentiation among populations of this native elm species. Overall, given the distribution of North American elms and their wind-pollination system, and based on genetic structure data previously collected in *U. rubra*, we expect little genetic differentiation among populations of the North American native elm species. We therefore expect much—perhaps most—of the genetic diversity of these native elm species to be maintained within a single or a few populations.

Selfing is low in the native U.S. elm species where it has been examined (Lester 1971). We also expect low levels of biparental inbreeding (matings among close relatives) given the potentially high gene flow via pollen in these highly outcrossed species. Although we do not have data on outcrossing rates for many North American elm species, we expect their populations to be strongly outcrossed and to have high levels of heterozygosity as was observed in *U. rubra* (Zalapa et al. 2010). In highly heterozygous populations, the total number of alleles can be recovered

in fewer individuals than for inbred populations. The number of individuals needed to recover all the alleles would be even less in tetraploid individuals of *U. americana* where each individual can carry up to four distinct alleles (Whittemore and Olsen 2011). Based on the mating system and pollen dispersal mechanism of these North American elm species, and on the results obtained from genetic studies of *U. rubra*, we predict little impact of DED on the level of genetic diversity within, and the pattern of distribution of genetic diversity among, populations of North American elm species. *Ulmus serotina* may be an exception if genetic differentiation is strong and populations are decimated by disease. Future genetic studies should test these predictions.

In previous studies, we detected frequent hybridization between *U. rubra* and *U. pumila* (Zalapa et al. 2009, 2010). Hybridization increased the genetic diversity of *U. pumila* populations and we hypothesized introgressed *U. rubra* genes and new gene combinations following hybridization may have facilitated adaptation of *U. pumila* to a wider range of environmental conditions (Zalapa et al. 2010). We expect *U.rubra-U.pumila* hybrids to be common where both species coexist and their number to increase over time. Moreover, given the observed introgression predominantly back to *U. pumila* (rather than *U. rubra)* the *U. rubra* genes are expected to mostly get eliminated over time in the hybrids (Zalapa et al. 2009). In addition, the directional pattern of introgression toward *U. pumila* suggests that DED resistance is unlikely to get transmitted to *U. rubra* in the wild. Hybridization and introgression back to *U. pumila* can therefore be added to forest fragmentation by humans and DED as factors affecting the long-term conservation of *U. rubra* in the United States.

The recent discovery of diploid *U. americana* trees throughout its range (Whittemore and Olsen 2011) raises the question of whether these diploid trees could also hybridize with *U. pumila*. If the incompatibility between *U. americana* and *U. pumila* resulted simply from differences in ploidy levels, with tetraploid *U. americana* not crossing with diploid *U. pumila*, then the diploid *U. americana* would raise concern about potential hybridization with *U. pumila.* However, other factors besides ploidy levels seem to contribute to the incompatibility observed between these two species (Ager and Guries 1982). The absence of morphological hybrids between *U. pumila* and *U. americana* across the landscape, despite the widespread range of *U. americana*, supports the presence of some incompatibility barrier between these two species (Ager and Guries 1982). This situation differs sharply from the extensive hybridization observed between *U. pumila* and *U. rubra* across the range of *U. rubra.* Based on the available evidence, we predict that *U. americana* is not likely to hybridize and introgress with *U. pumila* and we do not expect hybridization to negatively affect *U. americana*.

We hypothesize that hybridization between *U. pumila* and the other native elm species is more likely because no incompatibility barriers are known between these species. An older study reported mortality of seedlings from crosses between *U. pumila* and *U. thomasii* at the Lake States Forestry Experimental Station in the 1950s (Sholtz 1957). While this was attributed to "hybrid lethality," more research is needed to confirm whether pre- or post-zygotic barriers exist between these two elm species. Hybridization between *U. pumila* and four other native elm species (i.e., *U. thomasii*, *U. alata, U. crassifolia,* and *U. serotina*) may be more difficult to detect across the landscape given the smaller range of these native elm species relative to *U. rubra*. However, any hybridization between *U. pumila* and these wild elm species is likely to have negative effects and could engender conservation concerns (Ellstrand and Schierenbeck 2000, Rieseberg et al. 2003). The threat is greatest for small populations already at risk from other stresses where continued hybridization could lead to genetic assimilation and eventual loss of a native taxon (Prentis et al. 2007, Rhymer and Simberloff 1996). More studies are needed to determine the size and distribution of *U. serotina* populations because, given its most restricted range and the increased stress imposed by DED, this native elm species could be the most threatened by hybridization.

Conclusions

Despite the impact of DED, large numbers of elms survive to reach reproductive maturity, and as a result of their wind-pollination system generating strong gene flow and the fact that they are strongly outcrossed, we expect the disease not to have diminished the genetic diversity of North American native elm species. One exception could be *U. serotina* if strong fragmentation occurs among its scarcer populations. While hybridization is extensive between *U. rubra* and *U. pumila*, we do not expect much hybridization between *U. americana* and *U. pumila* because of incompatibility barriers, in addition to differences in ploidy levels. Hybridization between *U. pumila* and the other four native elm species is more likely because no incompatibility barriers have yet been identified. Although hybrids may be more difficult to detect across the landscape, because these species are not as common as *U. rubra*, hybridization could have negative impact on the genetic integrity of these native elm species. The greatest threat would be for *U. serotina* given its more restricted range. While DED may be unlikely to reduce the genetic diversity of North American native elm species, the planting of more *U. pumila* across the landscape, partly in response to DED, increases the probability of hybridization for five North American native elm species with potential negative impact on the long-term conservation of these native species. Taken together, our work emphasizes the need to understand the long-term impacts of an invasive disease on native species to help determine if any intervention is needed such as a conservation program or an extensive breeding program. If planting an exotic species or hybrids between a native and an exotic species in response to an invasive disease threat, we must understand the potential risks of hybridization with our native species as well as impacts of hybridization on the long-term conservation of our native species. Finally, hybridization could transfer resistance to the native species, and, in addition, because hybridization can increase genetic diversity and create new genotypes, it could facilitate adaptation over time to an invasive threat.

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Literature Cited

Ager, A.; Guries, R.P. 1982. **Barriers to interspecific hybridization in *Ulmus americana*.** Euphytica. 31: 909-920.

Bey, C.F. 1990. ***Ulmus americana*.** In: Burns, R.M.; Honkala, B.H., eds. Silvics of North America, vol. 2 hardwoods. Agric. Handb. 654. Washington, DC: U.S. Department of Agriculture, Forest Service: 801-807.

Bouzat, J.L. 2010. **Conservation genetics of population bottlenecks: the role of chance, selection, and history.** Conservation Genetics. 11: 463-478.

Brasier, C.M. 2000. **Intercontinental spread and continuing evolution of the Dutch elm disease pathogens.** In: Dunn, C.P., ed. The elms: breeding, conservation and disease management. Boston, MA: Kluwer Academic Publishers: 61-72.

Brunet, J.; Zalapa, J.E.; Guries R.P. 2016. **Conservation of genetic diversity in slippery elm (*Ulmus rubra*) in Wisconsin despite the devastating impact of Dutch elm disease.** Conservation Genetics. 17: 1001-1010.

Burczyk, J.; DiFazio, S.P.; Adams, W.T. 2004. **Gene flow in forest trees: how far do genes really travel?** Forest Genetics. 11: 179-192.

Ding, J.; Reardon, R.; Wu, Y.; Zheng, H.; Fu, W. 2006. **Biological control of invasive plants through collaboration between China and the United States of America: a perspective.** Biological Invasions. 8: 1439-1450.

Ellstrand, N.C.; **Schierenbeck, K.A. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants?** Proceedings of the National Academy of Sciences. 97(13): 7043-7050.

Excoffier, L.; Smouse, P.E.; Quattro J.M. 1992. **Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data.** Genetics. 131: 479-491.

Glémin, S.; Bazin, E.; Charlesworth, D. 2006. **Impact of mating systems on patterns of sequence polymorphism in flowering plants.** Proceedings of the Royal Society of London B. 276: 3011-3019.

Kartesz J.T. 2015 **North American plant atlas.** Chapel Hill, NC: The Biota of North America Program (BONAP). http://bonap.net/napa.

Leopold, D.J. 1980. **Chinese and Siberian elms.** Journal of Arboriculture. 6: 175-179.

Lester, D.T. 1971. **Self-compatibility and inbreeding depression in American elm.** Forest Science. 17: 321-322.

Loveless, M.D.; Hamrick, J.L. 1984. **Ecological determinants of genetic structure in plant populations.** Annual Review of Ecology and Systematics. 15: 65-95.

Natural Resources Conservation Service (NRCS). 2017. **The PLANTS database.** Greensboro, NC: U.S. Department of Agriculture, Natural Resources Conservation Service. http://plants.usda.gov.

Parker, G.R.; Leopold, D.J. 1983. **Replacement of *Ulmus americana* L. in a mature east-central Indiana Woods.** Bulletin of the Torrey Botanical Club. 110: 482-488.

Peakall, R.; Smouse, P.E. 2006. **GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research.** Molecular Ecology Notes. 6: 288-295.

Prentis, P.J.; White, E.M.; Radford, I.J.; Lowe, A.J.; Clarke, A.R. 2007. **Can hybridization cause local extinction: A case for demographic swamping of the Australian native *Senecio pinnatifolius* by the invasive *Senecio madagascariensis*?** New Phytologist. 176: 902-912.

Pritchard, J.K.; Stephens, M.; Donnelly, P. 2000. **Inference of population structure using multilocus genotype data.** Genetics. 155: 945-959.

Richardson, C.J.; Cares, C.W. 1976. **An analysis of elm (*Ulmus americana*) mortality in a second-growth hardwood forest in southeastern Michigan.** Canadian Journal of Botany. 54: 1120-1125.

Rieseberg, L.H.; Raymond, O.; Rosenthal, D.M.; Lai, Z.; Livingstone, K. [et al.]. 2003. **Major ecological transitions in wild sunflowers facilitated by hybridization.** Science. 301: 1211-1216.

Rhymer, J.M.; Simberloff, D. 1996. **Extinction by hybridization and introgression.** Annual Review of Ecology and Systematics. 27: 83-109.

Sholtz, H.F. 1957. **Silvicultural characteristics of rock elm (*Ulmus thomasii*).** Station Paper 47. St. Paul, MN: U.S. Department of Agriculture, Forest Service, Lake States Forest Experiment Station.16 p.

Slatkin, M. 1987. **Gene flow and the geographic structure of natural populations.** Science. 236: 787-792.

Smalley, E.B.; Guries, R.P. 1993. **Breeding elms for resistance to Dutch elm disease.** Annual Review of Phytopathology. 31: 325-352.

Ware, G.H. 1995. **Little-known elms from China: landscape tree possibilities.** Journal of Arboriculture. 21: 284-288.

Whittemore, A.T.; Olsen R.T. 2011. ***Ulmus americana* (Ulmaceae) is a polyploid complex.** American Journal of Botany. 98: 754-760.

Zalapa, J.E.; Brunet, J.; Guries, R.P. 2009. **Patterns of hybridization and introgression between invasive *Ulmus pumila* (Ulmaceae) and native *U. rubra*.** American Journal of Botany. 96: 1116-1128.

Zalapa, J.E.; Brunet, J.; Guries, R.P. 2010. **The extent of hybridization and its impact on the genetic diversity and population structure of an invasive tree, *Ulmus pumila* (Ulmaceae).** Evolutionary Applications. 3: 157-168.

AMERICAN ELM REINTRODUCTION

AMERICAN ELM CLONES OF IMPORTANCE IN DUTCH ELM DISEASE TOLERANCE STUDIES

Linda M. Haugen and Susan E. Bentz[1]

Abstract.—We present the background and characteristics of American elm clones that are commercially available or of interest in research on Dutch elm disease (DED) tolerance in the United States. The characteristics of interest include origin, ploidy level, whether available in nursery trade, evidence of DED tolerance, and other comments. The list includes 10 named commercially available cultivars, six additional named American elms of interest, and six numbered clones of interest.

Introduction

Group discussions were held during the 2016 American Elm Restoration Workshop, to share information about clones of American elm that have been tested for Dutch elm disease (DED) tolerance. As an outcome of those discussions, and with much further consultation of the literature, a listing has been compiled of clones commercially available or of other interest.

The purpose of this report is to provide a concise listing of the characteristics and background of these clones, as a resource for American elm researchers. The list includes both commercially available clones and clones that have been used in research on DED tolerance in the United States. Some of the selections listed are known to be susceptible, but are included because they have been used as susceptible controls, were at one time considered to be tolerant, or are important as parents of DED-tolerant clones. The first group is an alphabetical list of clones that are commercially available in the nursery industry, including some that have not been tested for DED. The second group is clones of interest, also ordered alphabetically. A third group of numbered clones follow. This is not an exhaustive list, but includes those for which we had information available at the time of publication.

The characteristics of interest that we have included are information on origin, ploidy level, availability in the nursery industry, evidence of DED tolerance, and other comments. When it was available, information on growth form, is included in the general comments section. Additional descriptions of growth form can be found for many of the cultivars in the "Manual of Woody Landscape Plants" (Dirr 2009). Note that in these groups, proper cultivar names are included in single quotations (e.g., 'cultivar name'). Other names or numbers by which a clone has been identified follow in parentheses. Often a clone was first known by an accession number, or other identity, prior to receiving a name. The abbreviation NA, in association with a number, indicates the National Arboretum accession number.

For the sake of consistency of terminology within this paper, only the term tolerance is used to describe the relative response of elms to the DED fungus, to reflect the fact that all *U. americana* clones can be infected by the disease, but tolerant clones often recover from infection. Some researchers prefer to use the term resistance for this. No American elm has yet demonstrated immunity to DED, and the level of tolerance demonstrated varies based on growing conditions, type of inoculum, method of inoculation, and a variety of other factors (see Beier et al. 2017, Flower et al. 2017, Haugen et al. 2017, in this proceedings).

[1] Plant Pathologist (LMH), U.S. Forest Service, Northeastern Area State and Private Forestry, 1992 Folwell Avenue, St. Paul, MN 55108; Horticulturist (SEB), USDA Agricultural Research Service, National Arboretum. LMH is corresponding author: to contact, call 651-649-5029 or email at lhaugen@fs.fed.us.

Commercially Available Clones

'Brandon'; 'Patmore'

Origin and Notes: Selected and propagated by R.H. Patmore from a native tree in Brandon, Manitoba, Canada, with an upright compact form (listed in Santamour and Bentz 1995). Trees are sold under both cultivar names, which may or may not be synonymous.

Ploidy: Presumed tetraploid.

Availablility: Available mainly in Canada. Nursery catalogs recommend it for locations without DED or with active DED sanitation programs.

Evidence of DED tolerance: No information available. Thought to be susceptible.

Other Comments: Included because it is fairly widely planted in Canada in places with no DED. Limited information on this cultivar is available on Wikipedia.

Colonial Spirit Elm® ('JFS-Prince II', Kuhar 2)

Origin and Notes: Discovered by Princeton Nursery as a surviving mature tree in New Jersey. Introduced under license by J. Frank Schmidt & Sons Co. after Princeton Nursery closed.

Ploidy: Tetraploid.[2]

Availability: Available through J. Frank Schmidt & Sons Co., Boring, OR.

Evidence of DED tolerance: Still under evaluation. Limited numbers were challenge inoculated by U.S. Forest Service (Flower et al. 2017, this proceedings).

Other Comments: Upright vase shape, somewhat narrow vase when young then widening and arching into a classic vase shape. Princeton Nursery preferred this cultivar to its own 'Princeton' cultivar because of better branching.[3]

Creole Queen™ (*Ulmus americana* 'UASNZ')

Origin and Notes: Selected in New Orleans, LA, by Select Trees.

Availability: Available through Select Trees, Inc., of Athens, GA.

Evidence of DED tolerance: No record of testing was available.

Other Comments: Promoted as having a tight branching upright form with high heat tolerance.

'Jefferson' (NPS 3-487, N3487, NA 62001)

Origin and Notes: Selected by H.V. Wester of the National Park Service from trees planted on the National Mall in Washington, D.C. (Sherald et al. 1994). Joint release by Agricultural Research Service (National Arboretum) and National Park Service in 2004.

Ploidy: Triploid (Sherald et al. 1994, Whittemore and Olsen 2011, Whittemore and Xia 2017)

[2] Unpublished data on file with Alan Whittemore, Botanist, USDA Agricultural Research Service, National Arboretum, Beltsville, MD.

[3] Personal communication via email from Keith Warren, retired Director of Product Development, J. Frank Schmidt and Son Co., P.O. Box 189, Boring, OR 97009.

Availability: Generally available.

Evidence of DED tolerance: Tested by inoculation and found to be tolerant. Tolerance to DED tested by twig-crotch inoculations (Sherald et al. 1994) and stem inoculations (Townsend et al. 2005).

Other Comments: Thought to be a hybrid between tetraploid American elm and a diploid parent, probably a diploid "American elm" as reported by Whittemore and Olsen (2011). Spreading crown habit at maturity, suitable for and used on the National Mall (Sherald 1993). Cultivar description is available at National Arboretum (http://www.usna.usda.gov/Newintro/Jefferson%20elm(FinalLR).pdf). Bud break commences earlier and holds leaves longer than typical American elm. Discussion at the American Elm Restoration Workshop indicated that some material thought to be 'Jefferson' was commercially distributed (including to the National Elm Trial) and later determined to be mislabeled material of an unknown tetraploid elm, probably 'Princeton'. The extent of this distribution of misidentified material is not fully known and has significant implications for tree maintenance needs. It is possible to determine whether elms are truly 'Jefferson' through growth characteristics (using identification keys like those shared on the University of Minnesota website at http://trees.umn.edu/elmid) or testing the ploidy (true 'Jefferson' is triploid).[4]

'New Harmony' (Amer. 680, NA 57844)

Origin and Notes: Original tree was found along Interstate 70 near Springfield, OH. First propagated in 1980 (Wall 2000). Selection made in Delaware, OH, for DED tolerance by A.M. Townsend and L.R. Schreiber. Released in1995 by Agricultural Research Service (National Arboretum).

Ploidy: Tetraploid (Whittemore and Olsen 2011).

Availability: Generally available.

Evidence of DED tolerance: Tested by inoculation. Found to be tolerant (Townsend and Douglass 2001, Townsend et al 1995, 2005).

Growth Form: Narrow crown with good branch structure.

Prairie Expedition® ('Lewis & Clark'; RFM-37)

Origin and Notes: Origin is along Wild Rice River southwest of Fargo, ND. Survivor elms were identified through a landowner survey. Released in 2004 by the North Dakota State University (NDSU) Research Foundation. Suitable trees were screened (Cheng et al. 1997).

Ploidy: Presumed tetraploid

Availability: Generally available.

Evidence of DED tolerance: Tested by inoculation. Found by NDSU to be tolerant to DED (NDSU 2016). For more information, see Capps (1997).

Other Comments: Form typical of American elm. NDSU Research Foundation has trademarked (not patented) and released this elm for commercial production. Information on licensing is available at http://www.ndsuresearchfoundation.org/prairie_expedition.

[4] Personal communications with Tom Zetterstrom, Founding Director of Elm Watch, Canaan, CT 06018; and Chad Giblin, Research Fellow, Department of Forest Resources, University of Minnesota, St. Paul, MN 55108.

'Princeton'

Origin and Notes: Selected in 1922 by Princeton Nurseries in New Jersey (Green 1964).

Ploidy: Tetraploid.[2]

Availability: One of the most widely commercially available DED-tolerant clones.

Evidence of DED tolerance: Tested by inoculation. Found by ARS to be tolerant to inoculation with DED fungus (Santamour and Bentz 1995; Townsend et al. 1995, 2005).

Other Comments: Some resistance to elm leaf beetle (Green 1964). Discussion at elm meeting indicated need for high level of maintenance pruning to maintain good crown structure.

'St. Croix' (US Plant Patent 20,097 P3)

Origin and Notes: Selected by Mark Stennes from an agricultural property along the St. Croix River, near Afton, MN. The original tree is a very large tree and is still living (Bliska et al. 2009). The patent is held by Chris Bliska and others, with a $2/tree royalty (Clayton 2014).

Ploidy: Tetraploid.[5]

Availability: Generally available.

Evidence of DED tolerance: Tested by inoculation. Found to be tolerant. Tested alongside Valley Forge elm; both became symptomatic, but survived when wild-type elms died (Bliska et al. 2009).

Other Comments: Bailey Nurseries in Newport, MN, is producing this elm (Clayton 2014). Described as spreading, vase-shaped crown when open grown (Bliska et al. 2009).

"Survivor Tree" (Survivor)

Origin and Notes: Surviving American elm from 1995 bombing site of the Murrah Federal Building in Oklahoma City, OK. The name implies nothing about disease resistance, but instead is a tribute to the tree's persistence on the site. (Note the use of double quotes rather than single quotes traditionally used for cultivar names.)

Availability: Generally available.

Evidence of DED tolerance: No record of testing was available.

Other Comments: Ramets and seedlings from this tree are distributed for memorial plantings. See Oklahoma City National Memorial and Museum (2017.)

'Valley Forge' (Amer. 3, NA 57842)

Origin and Notes: Seedling selection made in Delaware, OH, for DED tolerance by A.M. Townsend and L.R. Schreiber. Released 1995 by Agricultural Research Service (National Arboretum). Oral tradition says the original seed source is Nebraska (Wall 2000).

Ploidy: Tetraploid.[2]

Availability: Widely available.

[5] Unpublished data on file with Benjamin W. Held, University of Minnesota.

Evidence of DED tolerance: Tested by inoculation. Found to be tolerant. (Townsend and Douglass 2001; Townsend et al. 1995, 2005).

Other Comments: Has been used as a standard tolerant clone in many research studies. Propagates easily. Young tree requires significant management to produce a tree of useful form. Poor structural characteristics combined with rapid growth have made this cultivar unsuitable for many landscape situations (Costello et al. 2004).

Other Clones of Interest

American Liberty (Included W502, W503, W505, W507, W510, and M-8; also referred to as Liberty)

Origin and Notes: A collection of six clones selected by E. Smalley and R. Guries at the University of Wisconsin. Five of the clones were selected from 8000 progeny of crosses from clones originating in Wisconsin and Iowa and similar trees from Cornell University and U.S. Department of Agriculture. The sixth clone (M-8) originated as one survivor out of 1000 seedlings from Kansas in 1957. Commercial development was transferred to the Elm Research Institute (Smalley et al. 1993).

Ploidy: Presumed Tetraploid.

Availability: Available through the Elm Research Institute (ERI), Harrisville, NH. Not available through nurseries.

Evidence of DED tolerance: Tested by inoculation. See US PP6227 (Smalley and Lester 1988). W502 and W510 have performed particularly well in some DED tolerance trials (Smalley et al. 1993). In stringent trials conducted by the Agricultural Research Service, the American Liberty multi-clone did not demonstrate high tolerance to DED, but ERI did not disclose the identity of the trees that were provided for the trial (Townsend and Douglass 2001, Townsend et al. 1995).

Other Comments: These were the first commercially released products of a DED tolerance breeding program (Smalley and Lester 1988). Reported as highly susceptible to elm yellows in New York (Sinclair et al. 1994). Form was noted as vigorous upright main trunk in youth with older branches tending to become more horizontal at maturity (Smalley et al. 1993). Since this is a multiclone, there may be variation in characteristics depending on which clone is received.

'Augustine' (Augustine Ascending)

Origin and Notes: Originally selected in 1927 in Bloomington, IL (listed in Green 1964).

Ploidy: Tetraploid.[2]

Availability: Unknown.

Evidence of DED tolerance: Has proven to be susceptible to DED (Santamour and Bentz 1995, Townsend et al. 2005).

Other Comments: This cultivar is included because it was very commonly planted at one time and it has been tested alongside tolerant American elms.

'Delaware' (Delaware 2)

Origin and Notes: Selected during the 1940s by the Bureau of Plant Industry in New Jersey (Townsend 2000). Origin of seed was North Dakota. Ramets from original material are retained at USDA site in Delaware, Ohio, and the National Arboretum in Beltsville, MD.

Ploidy: Tetraploid.[2]

Availability: No longer sold commercially, but is available for research purposes. It was available in the past.

Evidence of DED tolerance: Tested by inoculation. Found to be tolerant (Townsend et al. 2005).

Other Comments: Original tree died in 1980 from unknown causes (Santamour and Bentz 1995). Significant information on the history of this tree is included in Santamour and Bentz 1995. 'Delaware' is used by U.S. Forest Service, Northern Research Station (NRS) Forestry Sciences Laboratory in a number of plantings. Used in crosses to produce DED-tolerant progeny (Slavicek and Knight 2011, Townsend et al. 2005). Wide spreading, somewhat shorter than typical American elm (based on observations of the trees of this clone that are planted on the National Mall in Washington, D.C.).

'Independence' (W510) (US Plant Patent 6227)

Origin and Notes: Patented tree, part of American Liberty multiclone. Originated from a controlled cross between 'Moline' (from Illinois) and W185-21 (Smalley and Lester 1988). The W-185 family of American elm was received by Wisconsin in 1959 as a shipment of 200 seedlings from the (now defunct) Inter-state Nurseries in Hamburg, Iowa.[6]

Ploidy: Presumed tetraploid.

Availability: Not available. Only obtainable as one of American Liberty multiclone

Evidence of DED tolerance: Tested by inoculation (Smalley and Lester 1988, Townsend et al. 2005). Found by University of Wisconsin to be tolerant to DED.

Other Comments: Among the clones included in the American Liberty multiclone, W510 demonstrates tolerance and is also easy to propagate (Smalley et al. 1993). Form was noted as traditional American elm architecture (Smalley et al. 1993), vase shape, dense foliage, and vigorous growth rate (Smalley and Lester 1988).

'Moline'

Origin and Notes: Originated as a wild seedling transplanted to Moline, IL, in 1903. Has been propagated since 1916 (Green 1964).

Ploidy: Presumed tetraploid.

Availability: Currently unknown.

Evidence of DED tolerance: Reported both as slightly resistant [tolerant] (Smalley et al. 1993) and highly susceptible (Gibbs et al. 1975).

[6] Personal communication from Raymond P. Guries, Professor Emeritus, Department of Forest and Wildlife Ecology, University of Wisconsin, Madison, Wisconsin 53706. Information was provided to Linda Haugen as notes during American Elm Restoration Workshop.

Other Comments: This is the female parent of 'Independence', developed by University of Wisconsin (Smalley and Lester 1988).

NPS 3-178 ("Washington")

Origin and Notes: Selected by H. V. Wester from among trees planted on the National Mall in Washington, D.C. "Washington" is not considered a valid cultivar name (Santamour and Bentz 1995) and therefore appears in double, rather than single quotation marks here.

Ploidy: Triploid (Whittemore and Olsen 2011)

Availability: Currently not available. At one time was sold through Princeton Nurseries as "Washington" (Santamour and Bentz 1995, Sherald 1993).

Evidence of DED tolerance: Experiments by the National Park Service in the1960s indicate this clone is DED tolerant. This clone demonstrated greater vigor, including seasonally earlier foliage development, than other clones (Wester 1972).

Growth Form: Spreading crown habit at maturity, suitable for use on the National Mall (Sherald 1993).

Other Numbered Clones

(None of these is available in the nursery trade.)

NA 57845

Origin and Notes: "A randomly selected American elm clone" (Townsend et al. 2005). Used as a fully susceptible control in many ARS and NRS studies.

Ploidy: Tetraploid.[2]

Evidence of DED tolerance: Tested by inoculation. Fully susceptible to DED (Townsend et al. 2005).

Other Comments: This clone has been referred to informally as "FUBAR", but this is not a valid name. It has been used as a susceptible control in many ARS and NRS studies. Inoculations were applied to replicate plantation trees at Glenn Dale, MD, and Delaware, OH. At the Ohio site, NA 57845 was more susceptible to DED than average unselected seedlings grown from seed obtained from F. W. Schumacher Seed Company (Sandwich, MA). Ramets of this clone were also found to be susceptible in a field test conducted at University of California-Davis prior to 2000. These trees were mistakenly labeled as 8630 and later revealed through DNA testing to be NA 57845.[7]

Growth Form: Poor form.

R18-2 (NA 57846)

Origin and Notes: Originally selected by Cornell University and the Boyce Thompson Institute. Was one of 11 survivors out of 21,000 seedlings screened (Townsend et al. 2005).

[7] Personal communication from Steven Eshita, retired microbiologist, U.S. Forest Service, Northern Research Station, Delaware, Ohio. Information was provided to Linda Haugen as notes during American Elm Restoration Workshop.

Ploidy: Tetraploid.[2]

Evidence of DED tolerance: Tested by inoculation. Found to be tolerant (Townsend and Douglass 2001; Townsend et al. 1995, 2005).

Other Comments: Susceptible to elm yellows (EY). Original tree succumbed to EY in 1979 (Smalley et al. 1993). This clone was a parent in some of the clones included in American Liberty (Smalley et al. 1993) and is planted in several NRS plantings in Wisconsin, Iowa, Minnesota, and Connecticut.

Growth Form: Well-formed, vase-shaped tree.

Amer 180 (NA 55342)

Origin and Notes: Survivor of disease epiphytotic from near Findlay, Ohio.

Evidence of DED tolerance: Tested by inoculation. Found to be somewhat tolerant (Townsend 2000; Townsend et al. 1995, 2005).

Other comments: Not vigorous slow-growing. Cuttings are difficult to root.[8]

Amer 190 (NA 63507)

Origin and Notes: Originated from controlled cross between 'Valley Forge' and 'Delaware' (Townsend et al. 2005).

Ploidy: Tetraploid.[2]

Evidence of DED tolerance: Tested by ARS in 1984. Found to be among the best progeny from this cross (Townsend 2000). Tested by inoculation and found tolerant (Townsend et al. 2005).

Amer 290 (NA 63508)

Origin and Notes: Originated from controlled cross between 'Valley Forge' and 'Delaware' (Townsend et al. 2005).

Ploidy: Tetraploid.[2]

Evidence of DED tolerance: Tested by ARS in 1984. Found to be among the best progeny from this cross (Townsend 2000). Tested by inoculation. Found to be tolerant (Townsend et al. 2005).

Amer 8630

Origin and Notes: Survivor from selections made by Roger Swingle and associates at the Columbus, OH, U.S. Forest Service laboratory in Delaware, OH. Was selected in Kentucky as a survivor of initial DED and EY epiphytotics (Smalley et al. 1993).

Evidence of DED tolerance: Has never been tested for DED tolerance.[9]

Other Comments: This cultivar is included because of its potential for resistance to elm yellows.

[8] Personal communication from Susan E. Bentz, Horticulturist, USDA Agricultural Research Service, U.S. National Arboretum, Beltsville, MD 20705.

[9] Personal communication from James M. Slavicek, Project Leader, U.S. Forest Service, Northern Research Station, 359 Main Road, Delaware, OH, 43015.

Literature Cited

Beier, G.L.; Held, B.W.; Giblin, C.P.; Blanchette, R.A. 2017. **Effects of inoculation timing on symptom development in *Ulmus americana* L.** In: Pinchot, C.C.; Knight, K.S.; Haugen, L.M.; Flower, C.E.; Slavicek, J.M., eds. Proceedings of the American elm restoration workshop 2016; 2016 October 25-27; Lewis Center, OH. Gen. Tech. Rep. NRS-P-174. Newtown Square, PA: U.S. Department of Agriculture, Forest Service Northern Research Station: 30-36.

Bliska, C.; Bliska, P.; Stennes, M.; Giblin, C., inventors. 2009 (June 16). **American elm tree named 'St. Croix'.** U.S. Patent 20097P3.

Capps, T.K. 1997. **Selection, propagation, and inoculation of American elms with potential Dutch elm disease resistance.** Fargo, ND: North Dakota State University. MS thesis.

Cheng, Z.-M.; Shi, N.-Q.; Herman, D.E.; Capps, T.K. 1997. **American elm: building in resistance to Dutch elm disease.** Journal of Forestry. 95(3): 24-27.

Clayton, C. 2014. **The St. Croix elm.** Mpls. St. Paul magazine. February 2014. Available at http://mspmag.com/arts-and-culture/general-interest/the-st-croix-elm/ (accessed June 2014).

Costello, L.R.; Scott, S.R.; Drake, C.M. 2004. **A 10-year evaluation of the performance of four elm cultivars in California, US.** Journal of Arboriculture. 30(2): 114-122.

Dirr, M.A. 2009. **Manual of woody landscape plants their identification, ornamental characteristics, culture, propagation and uses.** Champaign, IL: Stipes Publishing. 1005 p.

Flower C.E.; Slavicek, J.M.; Lesser, D.; Eshita, S.; Pinchot, C.C. 2017. **Canopy decline assessments in American elm after inoculation with three doses of *Ophiostoma ulmi* and *O. novo-ulmi*.** In: Pinchot, C.C.; Knight, K.S.; Haugen, L.M.; Flower, C.E.; Slavicek, J.M., eds. Proceedings of the American elm restoration workshop 2016; 2016 October 25-27; Lewis Center, OH. Gen. Tech. Rep. NRS-P-174. Newtown Square, PA: U.S. Department of Agriculture, Forest Service Northern Research Station: 24-29.

Gibbs, J.N.; Brasier, D.M.; McNabb, H.S., Jr.; Heybroek, H.M. 1975. **Further studies on pathogenicity in *Ceratocystis ulmi*.** Forest Pathology. 5(3): 161-174.

Green, P.S. 1964. Registration of cultivar names in Ulmus. Arnoldia. 24 (6-8): 41-80.

Haugen, L.M.; Beier, G.L.; Bentz, S.E.; Guries, R.P.; Slavicek, J. 2017. **Challenge inoculations to test for Dutch elm disease tolerance: a summary of Methods used by various researchers.** In: Pinchot, C.C.; Knight, K.S.; Haugen, L.M.; Flower, C.E.; Slavicek, J.M., eds. Proceedings of the American elm restoration workshop 2016; 2016 October 25-27; Lewis Center, OH. Gen. Tech. Rep. NRS-P-174. Newtown Square, PA: U.S. Department of Agriculture, Forest Service Northern Research Station: 37-42.

North Dakota State University Research Foundation (NDSU). 2016. **NDSU Research Foundation website for transfer of scientific discoveries to the marketplace.** http://www.ndsuresearchfoundation.org/prairie_expedition. (accessed November 30, 2016).

Oklahoma City National Memorial and Museum. 2017. **Survivor tree.** https://oklahomacitynationalmemorial.org/press-room/press-kit/survivor-tree/ (accessed June 8, 2017).

Santamour, F.S., Jr.; Bentz, S.E. 1995. **Updated checklist of elm (*Ulmus*) cultivars for use in North America.** Journal of Arboriculture. 21:122-131.

Sherald, J.L. 1993. **Demands and opportunities for selecting disease-resistant elms.** In: Sticklen, M.; Sherald, J., eds. Dutch elm disease research: cellular and molecular approaches. New York, NY: Springer-Verlag: 60-68.

Sherald, J.L.; Santamour, F.S., Jr.; Hajela, R.K.; Hajela, N.; Sticklen, M.B. 1994. **A Dutch elm disease resistant triploid elm.** Canadian Journal of Forest Research. 24: 647-653.

Sinclair, W.A.; Griffiths, H.; Lee, I-M. 1994. **Mycoplasmalike organisms as causes of slow growth and decline of trees and shrubs.** Journal of Arboriculture. 20(3): 176-189.

Slavicek, J.M.; Knight, K.S. 2011. **Generation of American elm trees with tolerance to Dutch elm disease through controlled crosses and selection.** In: Sniezko, R.A. [et al.], tech coords. Proceedings of the fourth international workshop on the genetics of host-parasite interactions in forestry: disease and insect resistance in forest trees. Gen. Tech. Rep. PSW-GTR-240. Albany, CA: US Department of Agriculture, Forest Service, Pacific Southwest Research Station: 342-346.

Smalley, E.B.; Guries, R.P.; Lester, D.T. 1993. **American Liberty elms and beyond: going from the impossible to the difficult.** In: Sticklen, M.; Sherald, J., eds. Dutch elm disease research: cellular and molecular approaches. New York, NY: Springer-Verlag: 26-45.

Smalley, E.B.; Lester, D.T., inventors. 1988 (July 19). **Elm tree named Independence.** U.S. Plant Patent 6227.

Townsend, A.M. 2000. **USDA genetic research on elms.** In: Dunn, C.R, ed. The elms: breeding conservation and disease management. Boston, MA: Kluwer Academic Publishers: 271-278.

Townsend, A.M.; Douglass, L.W. 2001. **Variation among American elm clones in long-term dieback, growth, and survival following *Ophiostoma inoculation*.** Journal of Environmental Horticulture. 19(2): 100-103.

Townsend, A.M.; Bentz, S.E.; Douglass, L.W. 2005. **Evaluation of 19 American elm clones for tolerance to Dutch elm disease.** Journal of Environmental Horticulture. 23: 21-24.

Townsend, A.M.; Bentz, S.E.; Johnson, G.R. 1995. **Variation in response of selected American elm clones to *Ophiostoma ulmi*.** Journal of Environmental Horticulture. 13: 126-128.

Wall, J. 2000. **Return of the native: The American elm finds tolerance for a dread disease.** Penn State Ag Science magazine. Summer/Fall 2000: 29-32. [profile for alumnus A.M. "Denny" Townsend].

Wester, H.V. 1972. **Investigation of American elm clons [sic] based on physiological vigor for crown resistance to Dutch elm disease.** Plant Disease Reporter. 56 (8): 725-727.

Whittemore, A.T.; Olsen, R.T. 2011. ***Ulmus americana* (Ulmaceae) is a polyploid complex.** American Journal of Botany. 98(4): 754-760.

Whittemore, A.T.; Xia, Z.-L. 2017. **Genome size variation in elms (*Ulmus* spp.) and related genera.** Hortscience. 52(4): 1-7.

REPORT FROM THE STREET: ELM REINTRODUCTION

Tom Zetterstrom[1]

Since the introduction of Dutch elm disease (DED) to North America, heritage elm preservation was paramount, but elm restoration is held as the long-term solution. Various elm restoration efforts have been advanced and encouraged over the past half century, and all have inspired the euphoria of the "Return of the American Elm." The changing cast of characters on the public stage of Main and Elm Streets has, however, been met with mixed reviews.

An evaluation of several generations of elm restoration reveals recurring shortcomings in DED tolerance and/or sustainable crown structure and associated maintenance demands. In at least one early case, stature was simply unable to rise to the reputation of the tree.

Initially, 'Buisman elm' (*U. minor* 'Christine Buisman'), though European, was the only elm restoration cultivar available, and was being planted as late as the 1970s in southern New England. Ultimately it lagged behind in size, looked unlike American elm in habit, and fell out of favor.

In the 1980s and 1990s, 'Liberty elm' (*U. americana* 'American Liberty') held sway, as a group of six cultivars, but was unreliable in terms of DED tolerance, and generally performed no better than background elms. Nonetheless, Elm Research Institute's promotion continued despite repeated losses to DED. Nineteen years after a 1997 community planting of 35 'Liberty elms' in Sheffield, Massachusetts, 60 percent have been lost to DED.

Elm Watch formed in 1999 to advocate for best elm preservation technology and to promote elm restoration with reliable elm cultivars based on National Arboretum test results. Our objective was to plant "hundred-year trees," the eventual successors to the Heritage elms we had protected. Due to the high visibility of prominent public plantings, we optimized tree pits, managed the trees over time, and became fully aware of the demands and disappointments of our good work. Comments on particular cultivars below are derived from steep learning curves in service to public trees within the tri-state area of Connecticut, Massachusetts, and New York and thus differ from performance data from hands-off elm plots of the National Elm Trial (NET; Colorado State University, n.d.).

Valley Forge (*U. americana* 'Valley Forge'). Though the top DED performer in 2001 National Arboretum tests (Townsend and Douglass 2001), 'Valley Forge' turned out to be a loser due to awkward branch angles projecting growth in every direction except upward. Even the most skilled and dedicated pruners can barely get the tree through its adolescence, and catastrophic branch failure becomes increasingly probable from year to year. Its very rapid growth rate exacerbates structural problems and the cultivar is not recommended.

Princeton (*U. americana* 'Princeton'). 'Princeton' was found to have a relatively high pruning requirement in the trials at the University of California at Davis (according to the Princeton elm Wiki page) and has moderate DED resistance, though greater susceptibility than would be expected based on a layman's interpretation of "survival" in 2001 National Arboretum test results (Townsend and Douglass 2001).

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Of some 100 elms planted from 2001 to 2007 within the tri-state area, nine came down with DED, seven died, and two were saved by assiduous pruning. If one can extrapolate 9 percent losses, from about a 12-year interval, one might be left with only a few "hundred-year trees."

Princeton's structural vulnerabilities were evident in multiple codominant leaders that needed regular pruning at short intervals, not to exceed 3 years, and preferably 2 years. Otherwise, bark inclusions in acute branch angles, flaunted by Princeton's characteristic and recurring multi-stem branching patterns (accurately referred to as "cluster crotches"), resulted in high wind vulnerability and increased potential for catastrophic failure. The optimal pruning technique, involving pruning from below as well as pruning from above, can result in good structure, but is rarely implemented in community forestry settings.

New Harmony (*U. americana* 'New Harmony'). 'New Harmony' has good structural potential, due to its tendency to form an upright dominant leader, but any competing leaders with narrow crotches will need reduction cuts or straightaway deletion. Several of the large New Harmony elms at the Northern Research Station were likely planted in tight plots so branch spread may not have been properly expressed. Those narrow crotches, however, were disquieting to observe and in a community planting would have begged early removal.

Jefferson Elm (*U. americana* 'Jefferson'). 'Jefferson' is the structural winner and requires one-tenth the pruning compared to Princeton. This triploid elm has a slower growth rate and excellent branch angles. DED tolerance was very high in 2005 National Arboretum test results (Townsend et al. 2005). To confirm DED tolerance, Jefferson elms of increased sizes should be reinoculated before one has full confidence to use it in high visibility public plantings. Consumers need to also be aware that a nursery trade mix-up a dozen years ago still plays out in the marketplace, and 'Princeton' elms continue to be sold as 'Jefferson' unknowingly by reputable nurseries from New York to Minnesota. The Princeton/Jefferson comparative elm identification guide is available online at trees.umn.edu/elmid.

Because of unresolved DED and elm yellows concerns, American elm cultivars are not recommended for more than singular plantings, according to Elm Watch and Bruce Fraedrich of Bartlett Tree Research Lab (Charlotte, NC). Allee plantings are inconsistent with current sustainable community forestry practices and should be understood as an old-school aesthetic lacking in the diversity required for future resiliency. Monoculture plantings, such as alongside Pennsylvania Avenue in Washington, D.C., have disproportionate vulnerabilities to disease, and because of a poor understanding of pruning goals, uniformity of streetscape design may likely become disrupted over time along that Inauguration Day parade route due to expected failures of major structural leaders.

National Elm Trial (NET) results were inconclusive and provided no data on 'Jefferson' due to the cultivar mix-up. Extending the utility of remaining elm plots nationally for further testing, including reinoculation of older elms, such as is planned at the University of Minnesota Elm Research Program, is needed to bring elms back with renewed confidence. The NET did provide evaluations of many elm hybrids, mostly of Asian ancestry, several of them performed very well, and a few can even masquerade as American elm in form, particularly Accolade and Triumph, though smaller in height. It could be argued that those hybrids make better choices for community forestry settings, while American elm research largely remains a work in progress.

Current National Arboretum research on diploid American elms is most intriguing and may introduce a new set of players on the elm stage. U.S. Forest Service research, using crosses of presumed "survivor elms" with elms of measurable DED tolerance may also produce positive results. These research efforts deserve extended funding and should resist the temptations

of premature conclusions and premature releases. Researchers must recognize that cultivars, once released to the trade, can hang around for decades, even if they turn out to be unworthy. Research efforts on both DED tolerance and structure should rather stretch out the testing and provide enough time and space to reveal crown development while creating opportunities for reinoculations at larger diameters, and using various protocols. Bruce Carley and other elm activists appreciate the imperfect elm cultivars as important stepping stones in a long-term process that is still evolving toward a desirable tree and are encouraged to see improved branch structure in crosses such as 'Valley Forge' × 'Princeton.'

Foremost, American elm is notable as a street tree, a public tree, or a commemorative tree, planted with full expectations of a high canopy and presumed longevity. Yes, street trees come and go due to frequently inadequate planting practices and urban abuses, but within that context an elm can only stand among the survivors if structural and DED deficiencies are more fully resolved. We cannot afford another tree with the structural half-life of a Bradford Pear (Valley Forge), nor one with pruning demands that can be expected to span 20 years (Princeton). It is hard to recall another tree that has spawned the publication of its own manual, "Pruning Young Elms: Guiding American, Asian, and Hybrid Elms to Stately Maturity" (Giblin and Gilman 2010). As a bottomland tree in a natural setting the elm can also take its place, but will only be successful there if it can resist unmanaged disease pressures and hold a snow load. But as with the proverbial "tree in the (bottomland) forest," it may less likely be heard than its more highly visible and audible urban cousins, were it to fall.

Literature Cited

Colorado State University College of Agricultural Sciences. N.d. **National elm trial.** Ft. Collins, CO: Colorado State University. http://bspm.agsci.colostate.edu/national-elm-trial/ (accessed November 2, 2016).

Giblin, C.P.; Gillman, J.H. 2010. **Pruning young elms: guiding American, Asian, and hybrid elms to stately maturity.** Minneapolis, MN: Urban Forestry and Horticulture Research Institute. 96 p.

Townsend, A.M. and Douglass, L.W. 2001. **Variation among American elm clones in long-term dieback, growth, and survival following *Ophiostoma* inoculation.** Journal of Environmental Horticulture. 19: 100-103.

Townsend, A.M.; Bentz, S.E.; Douglass, L.W. 2005. **Evaluation of 19 American elm clones for tolerance to Dutch elm disease.** Journal of Environmental Horticulture. 23: 21-24.

PRUNING CYCLES AND STORM DAMAGE: ARE YOUNG AMERICAN ELMS FAILING PREMATURELY?

Chad P. Giblin and Gary Johnson[1]

Abstract.—The use of Dutch elm disease-resistant elms as a common replacement tree in municipal planting schedules has amassed a large population of these trees in many cities throughout the eastern half of the United States. Reports from practitioners have suggested that this population is vulnerable to catastrophic losses due to severe canopy failures during wind-loading events and that American elm (*Ulmus americana*) selections 'Princeton' and 'Valley Forge' are chronically among the most damaged, which is a combination of poor structure and sheer numbers in the landscape. In this study, tree failures resulting from two storms occurring in 2015 (28 July) and 2016 (05 July) in Saint Paul, Minnesota, were examined. In both cases, young American elms were failing due to excessive canopy damage at a rate of two to three times the failure rates of other tree species in the same landscapes.

Introduction

The increasing popularity of trees like Valley Forge and Princeton American elm (*Ulmus americana* 'Valley Forge' and *U. americana* 'Princeton') and other disease resistant elms have resulted in their widespread planting over the last decade. Since this increase in planting, there have been frequent reports of premature canopy failures, usually resulting from storms and other loading events. Arborists, urban foresters, and city planners and concerned about these reports and have expressed interest in determining if these failures are a result of increasing planting frequency or other factors unique to these species.

Tree Failure Due to Structural Defects

Experienced arboricultural practitioners know that codominant stems with branch inclusions are a recipe for disaster. This is the case for most elms that are larger and faster growing than other species. Codominant stems usually result from the loss of a main leader due to damage or removal. This loss encourages the growth of two or more new leaders that are competing for the same space in the young tree's canopy. Modern nursery production practices favor the removal the top portion of a young leader to encourage more side branching and thus form a more attractive (albeit temporary and artificial) crown. As these branches continue to grow in diameter, their attachment points become compressed, poorly attached, and more prone to failure (Fig. 1). Identification and timely correction of these defects is necessary to avoid catastrophic tree losses, especially during loading events. A study conducted at the Bartlett Tree Labs (Smiley 2003) examined the relative strength of codominant stems harvested from red maple. After harvest, the mechanical force required to separate branch unions with and without inclusions was measured using a dynamometer. Results showed that the presence of a branch inclusion resulted in branch unions that were significantly weaker. One interesting outcome of this research is the discussion of union strength and its relationship to branch size. In this study, smaller diameter branches with included bark were found to be weaker than larger ones. In summary, Smiley suggests that all branch inclusions should be considered weak when compared to those without included bark and should be addressed quickly.

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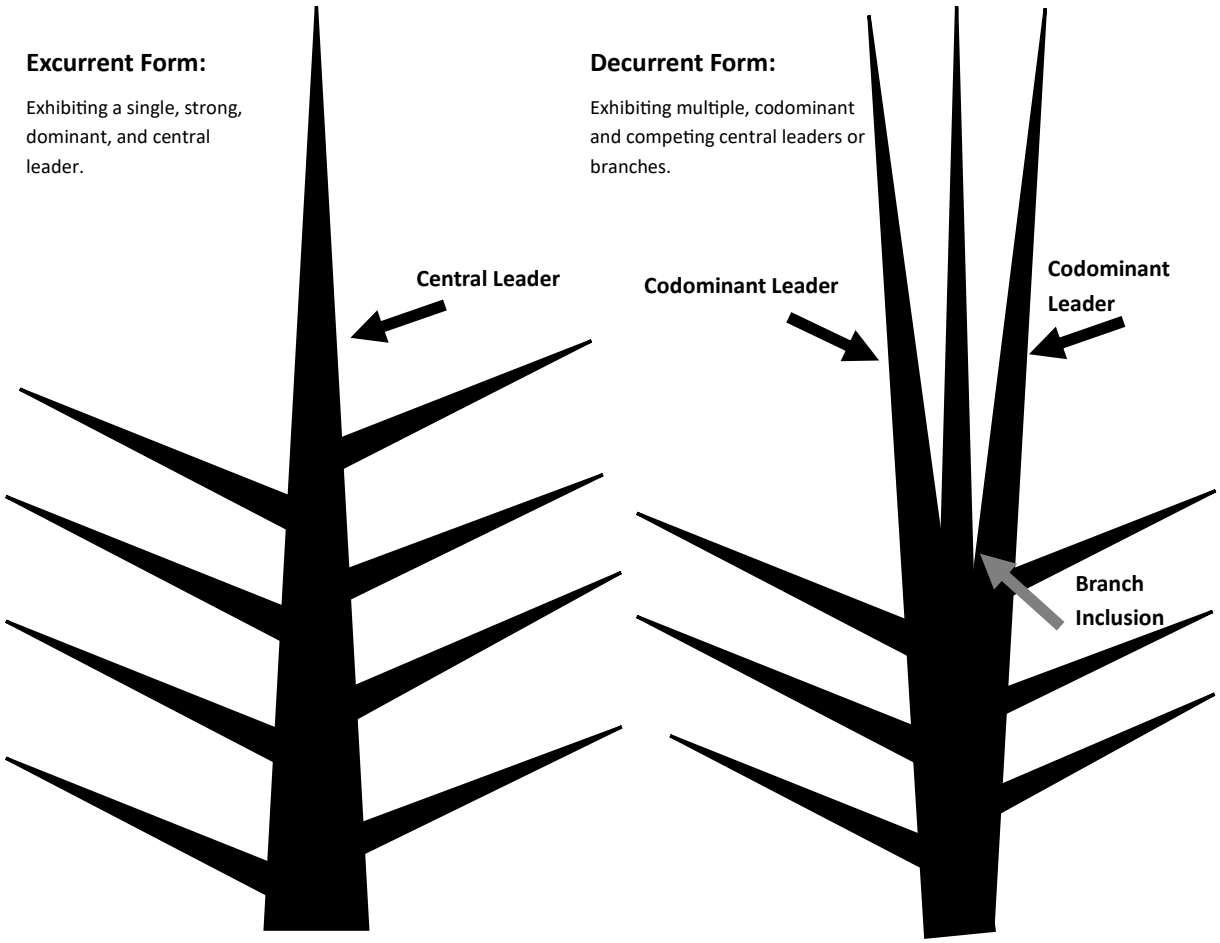

Figure 1.—Diagram of excurrent and decurrent growth forms showing codominant leaders and branch inclusions.

Gilman (2003) published results from a similar study that examined the role of branch-aspect ratio (BAR) in predicting branch union strength. BAR describes the size relationship between the diameter of branches and the diameter of the main stem at the point of attachment. BAR is typically reported with the size of the stem followed by the size of the branch. For example, a 2 cm branch attached to a 4 cm main stem has a BAR of 2:1. Gilman's work found that the amount of force required to break branches increases with BAR, stressing that codominant stems are much more likely to fail than other branches that are smaller in diameter. Gilman et al. (2015) published research that examined how suppression pruning cuts affect trunk strain at the point of branch attachment. Suppression cuts are a type of reduction pruning that removes a distal portion of a branch back to a lateral that is at least one-third but preferably one-half the size of the main branch. This is the first work focused on solving problems observed in the previous work (Gilman 2003). The effect of branch suppression was examined using pairs of codominant branches in live oak that were exposed to artificial wind loading events. To test the effects of reducing strain by pruning, the smaller of the two branches received one of four possible pruning doses that removed 0, 33, 66, and 100 percent of the leaves and branch tissue. They found that strain exerted on the branch attachment was reduced by increasing the pruning dose: more pruning caused a greater reduction in strain. Furthermore, the authors discuss the implications of using reduction pruning (i.e., suppression cuts) to reduce branch aspect ratio and thus increase the strength of the branch union. This allows for removal of multiple codominant branches staged over numerous pruning events, especially important when working with trees that have numerous defects or codominant branches.

Municipal Pruning Cycles: Cost-Benefit Analyses and the Price of Deferred Maintenance

During the last decade, there has been increased focus on developmental pruning of young trees. This practice is focused on guiding newly planted nursery stock from a period of intensive culture and very frequent pruning cycles to a much different maintenance regime in urban and community forests. Frequent pruning events during the first 15 years after planting should focus on the development and maintenance of a strong, central leader. Temporary branches that are located below the height of permanent, structural canopy in a tree should be managed using reduction cuts to suppress their growth to avoid removal at an excessively large size.

An emerging issue in municipal and commercial arboriculture and urban forestry is the cost of deferred maintenance. Miller and Sylvester (1981) examined this issue using Milwaukee, WI, as their subject city. The authors found that delaying maintenance resulted in trees of lower quality and, as a result, lower value. Because more frequent pruning cycles incur greater cost, the authors compared this decrease in value with the increased cost of more frequent maintenance. After statistical analysis, they determined that a pruning cycle between 4 and 5 years results in the best return from maintenance investments. In reference to small trees, the authors found a number of discrepancies in condition class during 1 year of their study and traced this back to a young tree population that needed "extensive corrective pruning...[resulting in] temporarily misshapen crowns, large pruning wounds, and a lower average condition class…". This is very interesting because it draws attention to the fact that these young trees needed major pruning, perhaps for the first time, implying that young trees are more sensitive to longer pruning cycles.

Ryder and Moore (2013) examined both the economic and biological effects of performing pruning on five species of trees. Their work compared the time required to perform developmental pruning on young trees (three times in 7 years) to that required when pruning older trees (one time after 20 years). In the case of eucalyptus, this delay increased the per-tree cost of pruning by 13 to 18 times. When inflation adjustments are made, the increase in time required may cost up to 25 times more than investing in developmental pruning of young trees. Another important point made was noting the decrease in tree defects when they received timely pruning as young trees. This creates an immediate savings in maintenance costs while reducing tree defects and subsequent storm damage linked to those defects. This may, in turn, decrease the overall pruning requirements of maturing trees and create a cost savings structure that lasts the lifetime of the tree.

The above research clearly supports the benefits of performing regular, developmental pruning on young trees. If this pruning is not performed, anecdotal information suggests that young trees—specifically young American elms—will fail at rates higher than other species. To test the hypothesis of exacerbated rates of failure in young elms, two wind loading events occurring in the Saint Paul, MN, area were examined.

Materials and Methods

Storm 1 occurred on 28 July 2015 and Storm 2 occurred on 05 July 2016. Storm damage and tree removal data was collected using Saint Paul tree inventory and work report information via Davey TreeKeeper® version 7 tree management software (Davey Resource Group, Kent, OH). The total number of trees requiring removal was determined for each storm and, in both storms, the rate of young elm failure was determined and compared to the rate of other species in these two storms. Failures were pooled at the genus level. Tree diameter at breast height (d.b.h.) (4.5 feet above the ground) was collected during post-storm surveys to assess damaged trees for removal. The breakdown of species and varieties within the elm genus was examined for both storms.

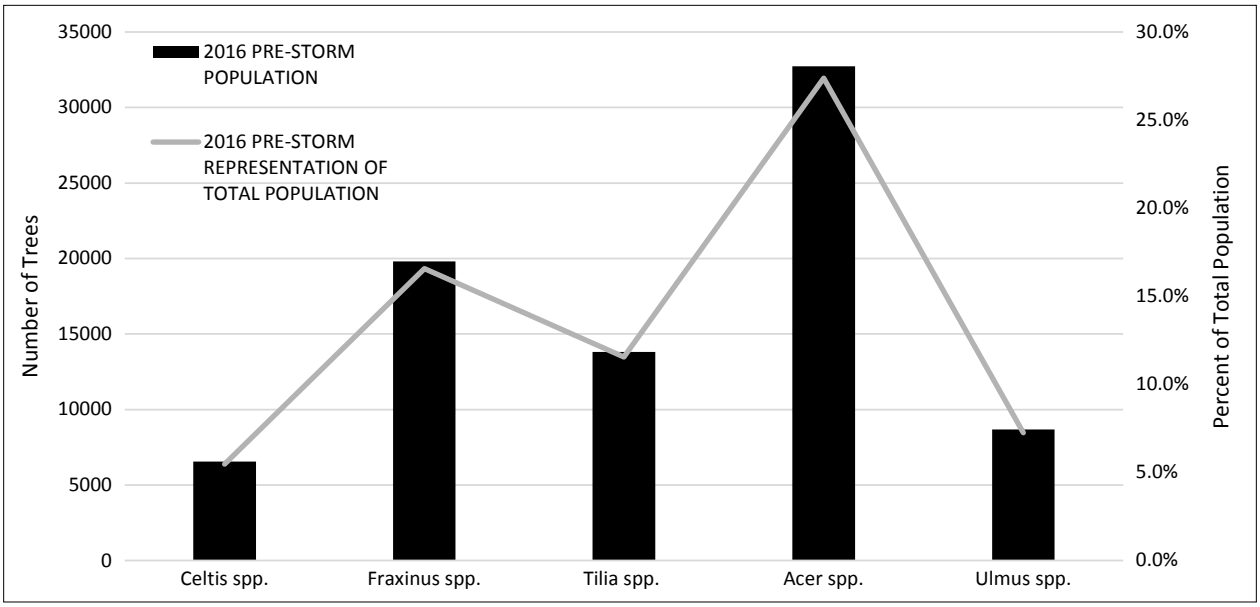

Figure 2.—Number of trees by genus present in Saint Paul, MN, prior to Storm 2 (05 July 2016) and the percentage of the total city tree population of each genus.

Five genera that exhibited storm-related failures at rates higher than other species in both storm events were selected for further study: ash (*Fraxinus* spp.), elm (*Ulmus* spp.), hackberry (*Celtis* spp.), linden (*Tilia* spp.), and maple (*Acer* spp.) This representation of genera is similar to data collected after other wind-loading events in the region (Johnson 2014). Citywide prestorm inventory data was available for Storm 2 in 2016, but not for Storm 1 in 2015. The total population of these five genera and their percent representation of the total population is shown in Figure 2.

Statistical frequency and chi-square tests were conducted to report species percentages, failure rates, and differences between failure rates in different genera and elm cultivars, varieties, and species using IBM SPSS Statistics version 22.0 (IBM 2013).

Results

Local weather data indicates that about 0.5 inches of rain fell during Storm 1 with maximum wind speeds of 25 miles per hour (m.p.h.) and gusts of 33 m.p.h. During Storm 2, about 1.0 inch of rain fell with maximum wind speeds of 38 m.p.h. and gusts of 67 m.p.h. After Storm 1, city staff assessed 181 trees for removal. Damage or failure resulting from Storm 2 required removal of 543 trees. Removal causes included catastrophic failure or irreparable damage to the crown due to codominant and/or included leaders and/or branches; failure due to windthrow; and stem failure.

For Storms 1 and 2, chi-square test procedures were used examine removals at the genus level and to test the null hypothesis that failure rate is equal across all genera. Test results indicate that removal rate was not consistent across all genera for either Storm 1 ($\chi^2(11, N=181) = 298.93, p < 0.01$) or Storm Two ($\chi^2(4, N=543) = 209.155, p < 0.01$). Five genera consistently exhibited storm-related failures at rates higher than other species in both storm events. Ash, elm, hackberry, linden, and maple represented approximately 90 percent of all failures in both storms, while these same five genera comprise just under 70 percent of the overall tree population citywide (Figs. 3 and 4).

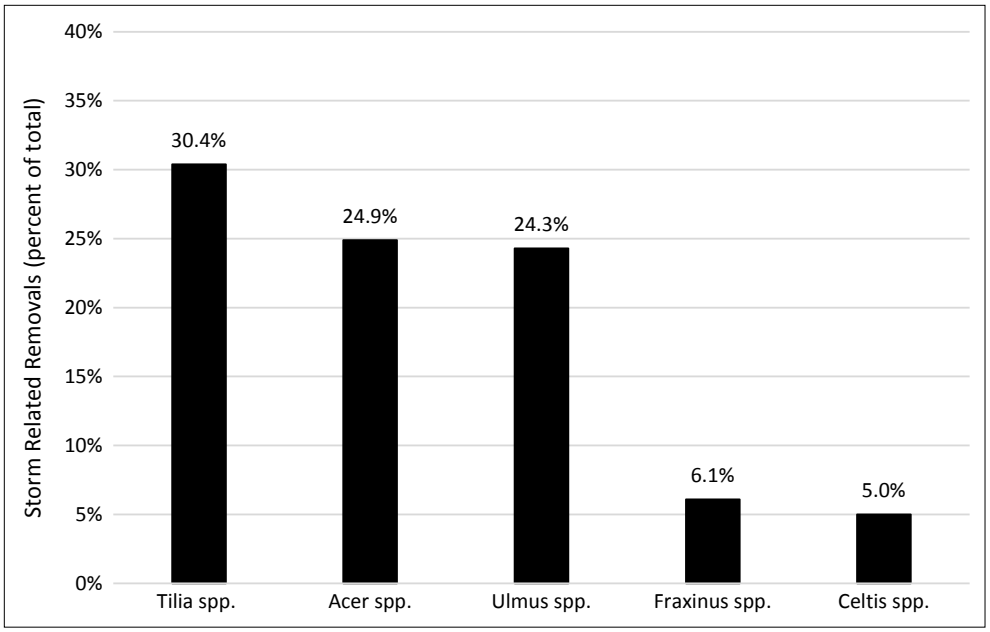

Figure 3.—Percentage of total storm-related removals by genus resulting from Storm 1 (28 July 2015).

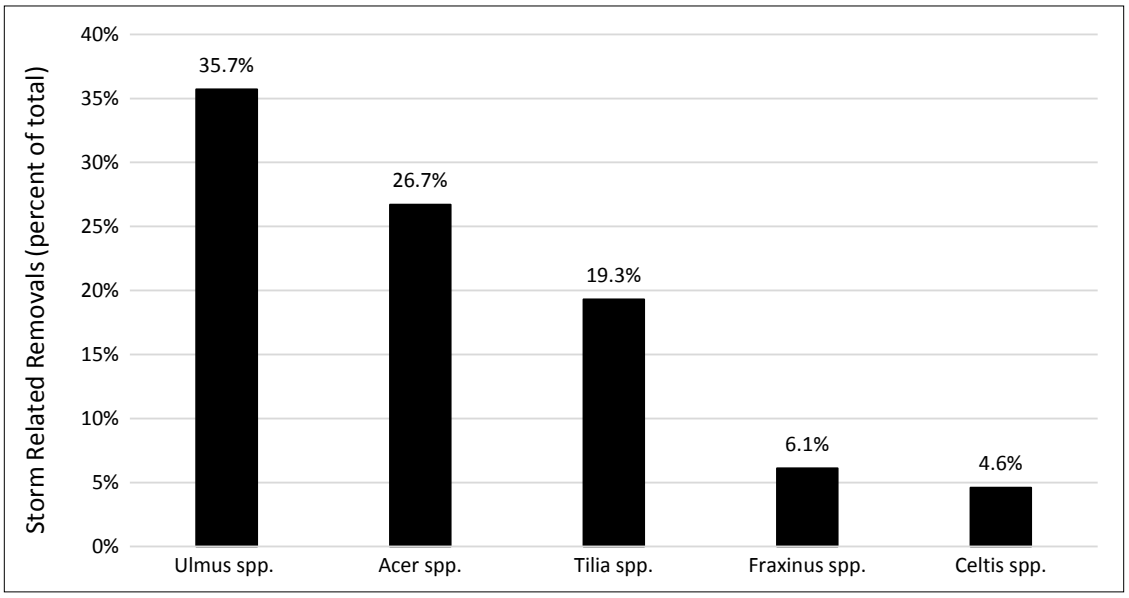

Figure 4.—Percentage of total storm-related removals by genus resulting from Storm 2 (05 July 2016).

In Storm 1, ash were largest trees at the time of failure averaging 19.1 inches d.b.h., followed by linden and maple at 18.2 inches d.b.h. and 15.1 inches d.b.h., respectively. Hackberry and elm were smallest at the time of failure at 13.1 inches d.b.h. and 5.5 inches d.b.h., respectively (Fig. 5). In Storm 2, linden and ash were the largest at the time of failure at 21 inches d.b.h. and 20.8 inches d.b.h., respectively, followed by maple at 16.9 inches d.b.h. Hackberry and elm were the smallest at the time of failure at 13.1 inches d.b.h. and 5.5 inches d.b.h. respectively (Fig. 6). Prestorm inventory data collected in 2016 shows that damage resulting from Storm 2 required the removal of 2.2 percent of the total elm population and less than 1 percent each of hackberry, ash, linden, and maple (Fig. 7). Total prestorm population percentages of these five genera were also calculated. Maple was the most populous at 27.4 percent, followed by ash and linden at 16.6 percent and 11.6 percent, respectively. Elm and hackberry were the least populous of these five genera at 7.3 percent and 5.5 percent, respectively (Fig. 7).

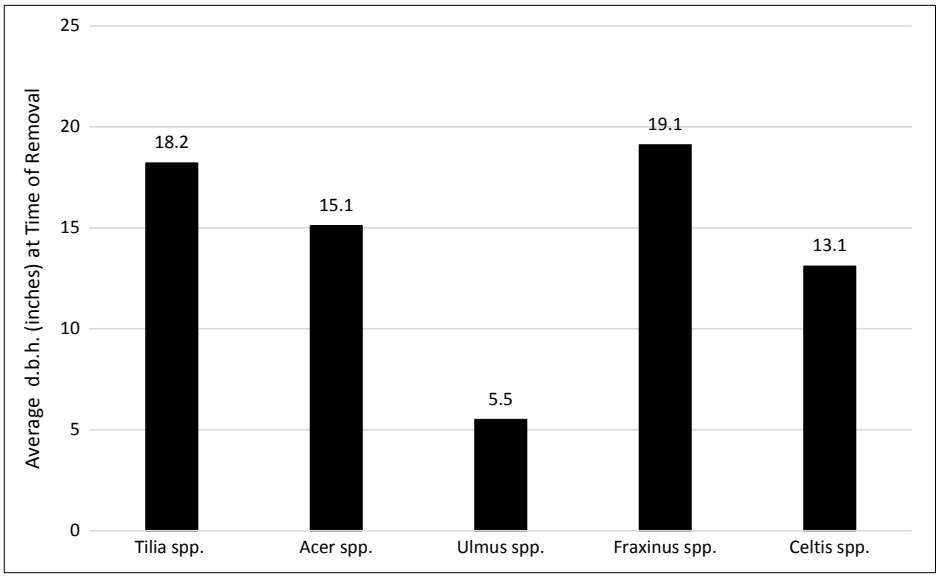

Figure 5.—Average d.b.h. at the time of removal after Storm 1 (28 July 2015).

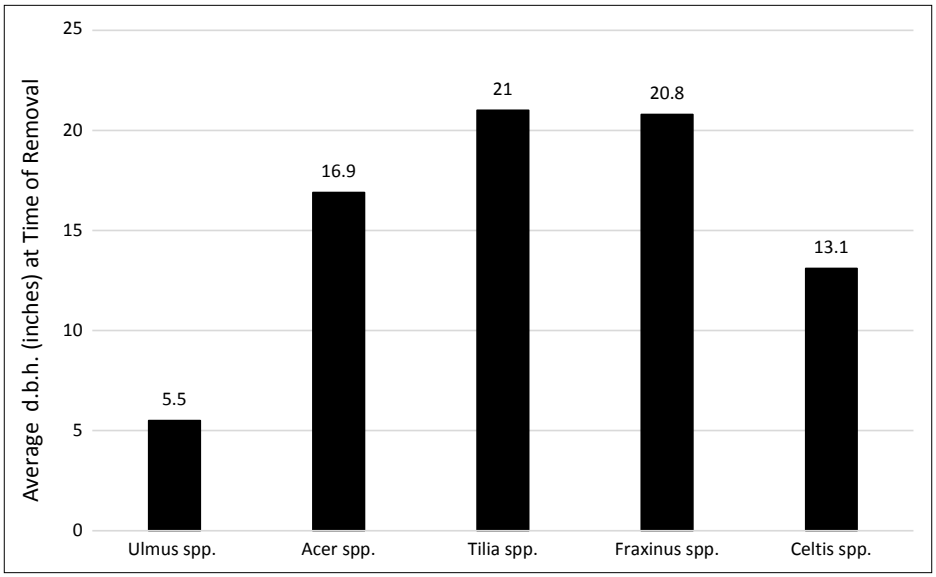

Figure 6.—Average d.b.h. at the time of removal after Storm 2 (05 July 2016).

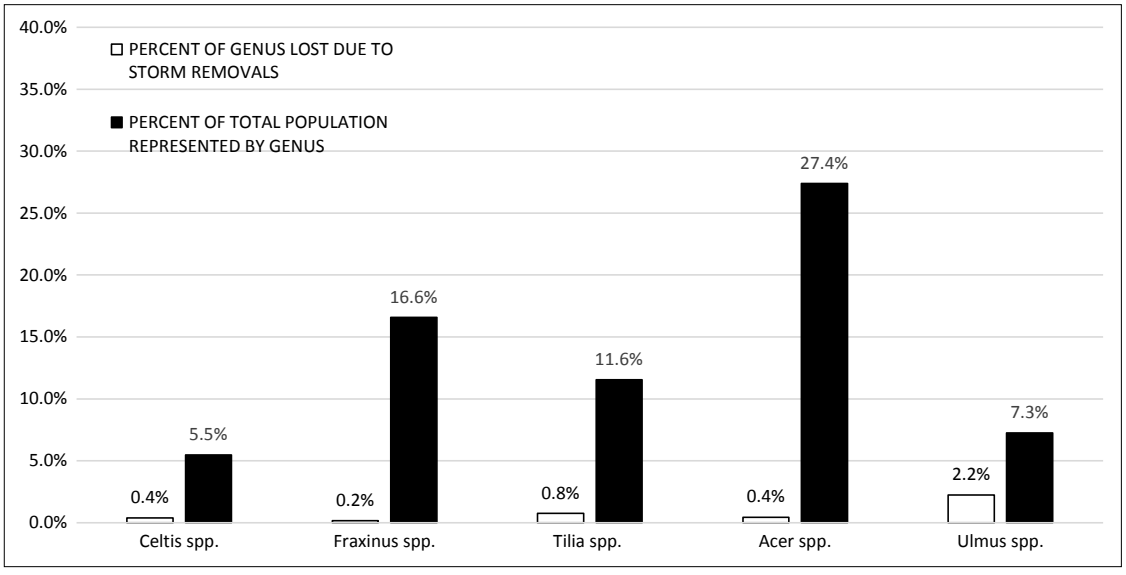

Figure 7.—Percentage of trees by genus lost due to storm removals after Storm 2 (05 July 2016) compared to the percent of the total city tree population of each genus.

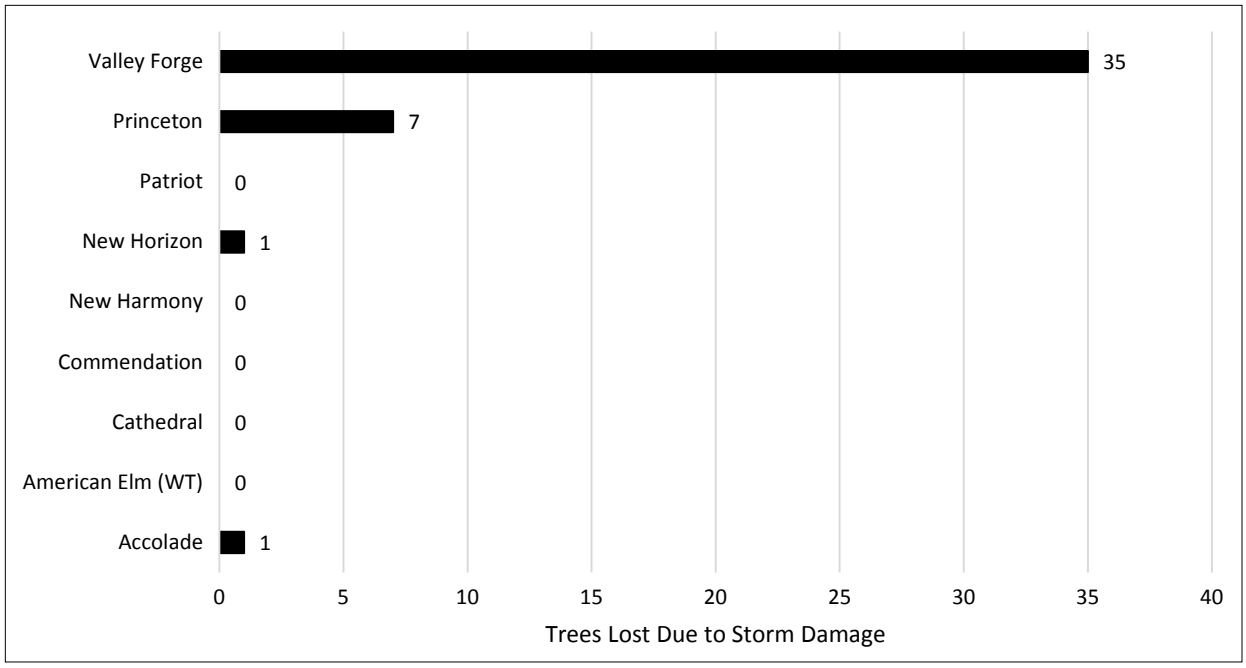

Figure 8.—Number of trees removed by elm species or variety resulting from Storm 1 (28 July 2015). WT refers to wild-type American elm.

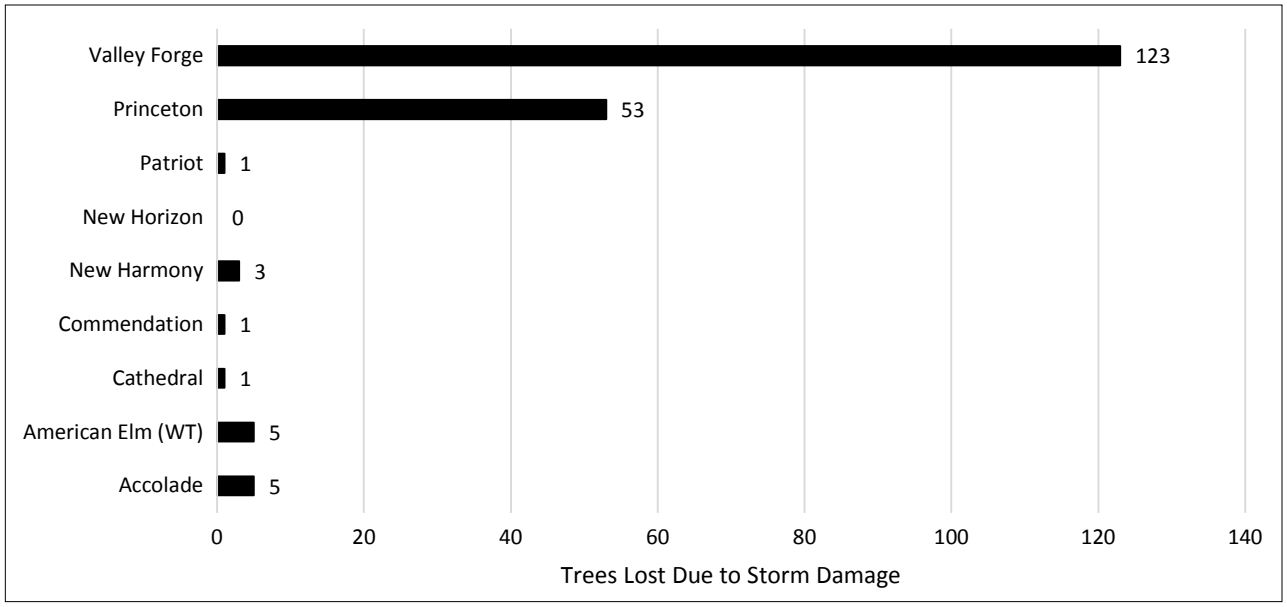

Figure 9.—Number of trees removed by elm species or variety resulting from Storm 2 (05 July 2016). WT refers to wild-type American elm.

In Storm 1, Valley Forge American elm failed more frequently than any other elm variety, cultivar, or species, with 35 removals. This was followed by Princeton American elm at seven removals (Fig. 8). The same trend was observed in Storm 2 with Valley Forge having the highest rate of failure within the elm genus at 123 removals and Princeton at 53 removals (Fig. 9). Varietal failure rates were calculated for Storm 2 only with 17.7 percent of all Valley Forge and 2.7 percent of all Princeton requiring removal after this storm (Figs. 10 and 11).

For both storms, chi-square test procedures were used examine removals at the cultivar, variety, and species level within the elm genus. Test results indicate that removal rate was

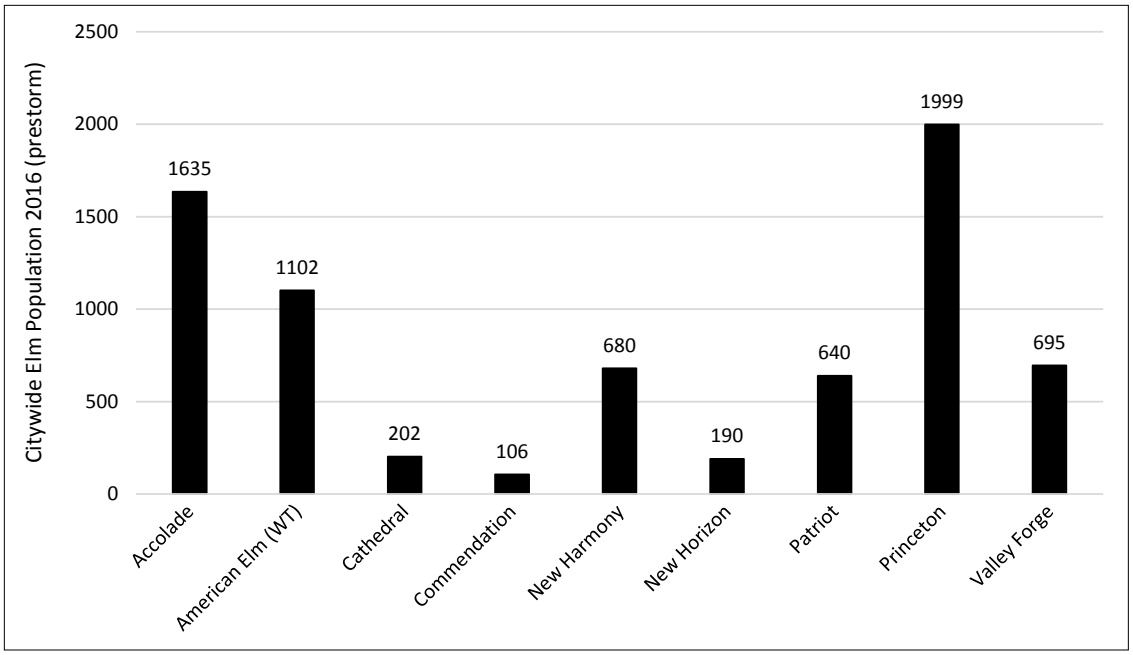

Figure 10.—Total elm population in Saint Paul, MN, by species or variety prior to Storm 2 (05 July 2016). WT refers to wild-type American elm.

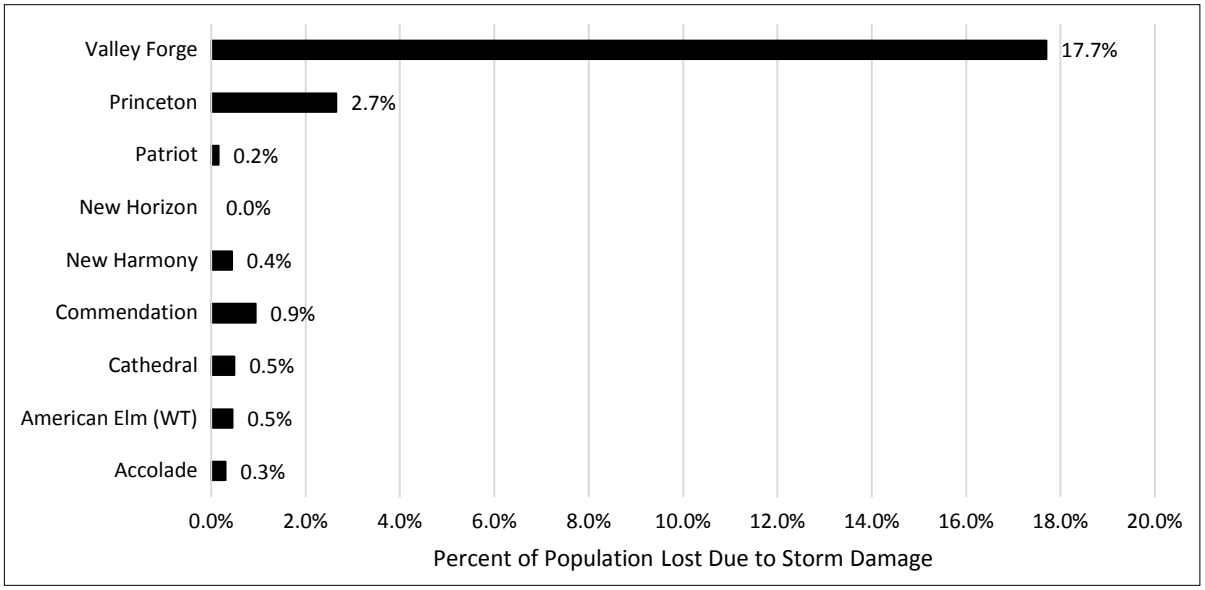

Figure 11.—Percentage of total elms removed by species or variety after Storm 2 (05 July 2016). WT refers to wild-type American elm.

significantly different between Princeton, Valley Forge, New Horizon, and Accolade elm in Storm 1 ($\chi^2(3, N=44) = 72.00, p < 0.01$). In Storm 2 removal rates also differed significantly between New Harmony, Princeton, Valley Forge, wild-type American elm, Accolade, Cathedral, Commendation, and Patriot elms ($\chi^2(9, N=192) = 733.94, p < 0.01$). Chi-square tests procedures were also conducted with adjusted expected removal values for Storm 2. Expected values were adjusted to account for overall representation of a cultivar, variety, or species within the elm genus and removal rate was also found to be significantly different between New Harmony, Princeton, Valley Forge, wild-type American elm, Accolade, Cathedral, Commendation, and Patriot elms ($\chi^2(7, N=192) = 676.04, p < 0.01$). Actual values, expected values, and residuals for elm removals resulting from Storm 2 are were also calculated (Table 1).

Table 1.—Total elm population in Saint Paul, MN, by species or variety prior to Storm 2 (05 July 2016). Actual losses resulting from storm-related removals and the predicted losses based on weighted frequencies and residuals determined by the overall representation of each species or variety within the elm (*Ulmus* spp.) genus. WT refers to wild-type American elm.

Elm Variety or Cultivar	Total Population 2016	Actual N	% of Population	Predicted N
Accolade	1635	5	22.6%	43
American elm (WT)	1102	5	15.2%	29
Cathedral	202	1	2.8%	5
Commendation	106	1	1.5%	3
New Harmony	680	3	9.4%	18
Patriot	640	1	8.8%	17
Princeton	1999	53	27.6%	53
Valley Forge	695	123	9.6%	18
	7059	192		187

Elm Variety or Cultivar	Total Population 2016	Actual (N)	Predicted (N)	Residual (N)
Accolade	1635	5	43	-39
American elm (WT)	1102	5	29	-25
Cathedral	202	1	5	-4
Commendation	106	1	3	-2
New Harmony	680	3	18	-16
Patriot	640	1	17	-17
Princeton	1999	53	53	-2
Valley Forge	695	123	18	104
TOTAL	7249	192		

Discussion

This initial examination of failure from two recent storms in Saint Paul, MN, indicates that elms, particularly young American elms, are suffering damage and subsequently, removal, at a rate that is disproportionate to their representation in the overall urban tree population of this city. These storm failures and removals that are observed in young American elms also are occurring much earlier in their lives than any other tree species. This trend is troubling when communities like Saint Paul are devoting so much effort to pruning young elms and other small trees. Saint Paul currently prunes young elms a minimum of three times during the first 10 years after planting. This pruning cycle is generally more frequent than other, similar communities and well-aligned with recommended pruning cycle frequencies reported in a recent review of literature (Vogt et al. 2015).

Additionally, there are two trends observed when elm failures are compared with other species that suffered damage and failures. First, elms are failing at a rate that is two or three times greater than other species when compared to their representative population, citywide. In Storm 2, elms lost 2.2 percent of their population while maples lost just 0.4 percent. A second trend observed in elms is their size at the time of failure. In both the 2015 and 2016 storms, the average d.b.h. at the time of failure for all elms was about 5.5 inches d.b.h., this even includes the handful of larger, mature elms that also failed. In contrast, all other species were much larger

at the time of failure, ranging from 13 inches d.b.h. in hackberry to nearly 20 inches d.b.h. in ash and linden. The other species typically failed due to wood decay at the time of failure while elm failures occur at weak branch or leader attachments in otherwise healthy, nondecayed wood tissue (Johnson, unpublished data). The observed form and exceptional growth rate of both Valley Forge and Princeton American elm may play a role in their exacerbated rate of storm damage and catastrophic failure. Both of these selections have an alarming tendency to form weak branch attachments at much more acute angles than other elm varieties—especially those of Asian origin. Additionally, American elms, in general, are less likely to exhibit an excurrent growth habit (i.e., a growth form with a strong, dominant central leader) (Fig. 1). Asian elms such as Accolade may possess inherent growth habits that are structurally superior to others.

The rate of failure observed in both Valley Forge and Princeton American elm indicates that more research is required to accurately determine optimum pruning cycles for these and other, similarly structured young trees in the municipal setting and to make recommendations for species that are properly aligned with expectations and municipal budgets allocated for young tree maintenance. Juvenile form of young elms is very plastic and can be greatly influenced—positively and negatively—in the production nursery setting. To avoid the release of elm cultivars that exhibit poor branch structure thus increasing likelihood of failure and damage during wind-loading events, researchers should conduct long-term growth habit assessments alongside Dutch elm disease resistance screening trials, preferably in cooperation with arboricultural researchers or arborists skilled in assessing juvenile form and pruning schedules. Also, because juvenile growth rates and forms of elm are so different than their mature counterparts, assessment of mature tree branch architecture (e.g., branch angle measurement and incidence of dysfunction) does not present itself as a technique useful for screening.

Acknowledgments

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Literature Cited

Gilman, E.F. 2003. **Branch-to-stem diameter ratio affects strength of attachment.** Journal of Arboriculture. 29(5): 291-294.

Gilman, E.F.; Miesbauer, J.W.; Masters, F.J. 2015. **Structural pruning effects on stem and trunk strain in wind.** Arboriculture & Urban Forestry. 41(1): 3-10.

IBM Corp. 2013. **IBM SPSS statistics for Windows, version 22.0.** Armonk, NY: IBM Corp.

Johnson, G.R. 2014. **When winds and trees collide: The influence of sidewalk repair, trunk flare, and wind-loading events on boulevard tree failures.** Arborist News. December 2014: 50-53.

Miller, R.W.; Sylvester, W.A. 1981. **An economic evaluation of the pruning cycle.** Journal of Arboriculture. 7(4): 109-112.

Ryder, C.M.; Moore, G.M. 2013. **The arboricultural and economic benefits of formative pruning street trees.** Arboriculture & Urban Forestry. 39(1): 17-24.

Smiley, E.T. 2003. **Does included bark reduce the strength of codominant stems?** Journal of Arboriculture. 29(2): 104-106.

Vogt, J.; Hauer, R.J.; Fischer, B.C. 2015. **The costs of maintaining and not maintaining the urban forest: A review of the urban forestry and arboriculture literature.** Arboriculture & Urban Forestry. 41(6): 293-323.

AMERICAN ELM (*ULMUS AMERICANA*) IN RESTORATION PLANTINGS: A REVIEW

Kathleen S. Knight, Linda M. Haugen, Cornelia C. Pinchot, Paul G. Schaberg, and James M. Slavicek[1]

Abstract.—The development of disease-tolerant American elm (*Ulmus americana*) trees has led to a need for reintroduction and restoration methods for the species. Here we review the current state of experimental work to inform reintroduction biology and restoration ecology of American elm. Much of this work is ongoing, and within several years the results will provide guidance for managers to use the species in restoration plantings. We identify additional research needs and opportunities to consider in development of American elm restoration strategies.

Introduction

Pests and pathogens have caused massive mortality events in multiple tree species around the world. The American chestnut (*Castanea dentata* [Marsh.] Borkh.), American beech (*Fagus grandifolia* Ehrh.), hemlock (*Tsuga canadensis* L. Carr.), butternut (*Juglans cinerea* L.), North American ash species (*Fraxinus* spp.), and American elm (*Ulmus americana* L.) are iconic species of the forests of the eastern and midwestern United States whose populations have been greatly affected (Wheeler et al. 2015). As new exotic insects and diseases continue to emerge through accidental introductions, additional tree species will be threatened. As trees succumb to these threats, the ecosystem services provided by forests will also be affected.

To respond to these threats, government, university, and nonprofit groups have led efforts to select and breed trees with resistance or tolerance to pests and pathogens (reviewed in Wheeler et al. 2015). Programs for different tree species are in varying stages of development and progress, depending on how long the effort has been ongoing and the particular challenges of the system. These tree improvement programs are critical to species adaptation to long-term threats to forest health (Wheeler et al. 2015). Many efforts share the goal of producing a genetically diverse suite of trees with tolerance to specific pests or pathogens, which then may be used in plantings in urban and natural areas. Restoration strategies and silvicultural requirements of the species will need to be determined in order to successfully establish founder populations of these species in natural areas. Once genetically diverse material and planting strategies are developed, operational reintroduction paired in some cases with ecosystem restoration should occur.

As efforts to restore American elm move forward, reintroduction strategies and silvicultural requirements are being tested. This review summarizes the ongoing efforts in testing restoration strategies for American elm and identifies gaps and opportunities in the restoration effort. The program to restore American chestnut provides a useful comparison for American elm restoration, as the work on American chestnut is in later stages. As the goal of developing blight-resistant American chestnut seedlings neared achievement (Steiner et al. 2017), work to understand the silvicultural requirements of the species and develop management strategies was initiated to improve reintroduction success.

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The biological characteristics of American chestnut, including response to light, soil nutrients and moisture, and competition are being considered with respect to operational planting strategies (Jacobs 2007). Knowledge of these species characteristics are necessary to select appropriate silvicultural and site preparation strategies (Clark et al. 2014). Test plantings of American chestnut have examined different planting methods and stock types in different climatic regions (Clark et al. 2014). These experiments have been valuable in identifying barriers to survival and establishment, primarily involving white-tailed deer (*Odocoileus virginianus*) browsing, infection by the root-rot pathogen (*Phytophthora cinnamomi*), and insect damage, but also including damage from *Pythium* root rot and drought. Planting high quality stock may be an important approach for overcoming these barriers (Clark et al. 2014). Researchers have identified pathways by which abiotic environmental conditions will affect biotic factors, including deer, disease, and insect damage, which will interact with the genetic makeup and the quality of tree seedlings to ultimately affect performance in the field (Clark et al. 2014).

American chestnut researchers also took a critical look at their program and identified gaps, opportunities, and challenges encountered by the restoration effort. Consideration of both the social and ecological contexts of programs is also important (Jacobs 2007, Jacobs et al. 2013). Understanding the social context guides the formulation of restoration planting goals and allows identification of policy, economic, or social barriers or constraints. Additional gaps in the ecological context, including seed zone testing, the incorporation of different sources of resistance, and combating invasive exotic insects were identified as areas for additional study (Clark et al. 2014). The long-term impacts and dynamics of reintroduction on forest ecosystems were considered, including questions about how plantings including American chestnut would be managed over time and how the species may spread from plantings (Jacobs 2007). Potential future challenges, including deployment strategies, were identified (Jacobs 2007).

Natural populations of American elm were greatly reduced due to the invasive fungal pathogens *Ophiostoma ulmi* and *O. novo-ulmi* that cause Dutch elm disease (DED), causing shifts in species composition within forest ecosystems (Barnes 1976). Indeed, DED remains an important force causing mortality of larger elms (Marks and Canham 2015). As a result, the role of American elm as a canopy species has been greatly reduced in forest ecosystems, and only smaller elms are commonly found (Marks 2017, Marks and Canham 2015). The development of DED-tolerant American elm selections, and the continued work to produce additional DED-tolerant selections, has generated considerable interest in the restoration of American elm. Research to better understand the ecology of the DED pathogen, tolerance of American elm selections, and effects of other serious pathogens (including elm yellows) is foundational to successful restoration plantings. The ultimate goal is to generate a diverse group of American elm selections with durable tolerance to disease. In addition to producing disease tolerant plants, researchers are testing restoration uses and methods for American elm. The combination of the appropriate planting stock and the knowledge of how to best use it will provide useful tools for managers.

Here we review the current experimental work both on reintroduction methods for American elm and on the use of American elm as part of a restoration strategy. The goals of reintroduction biology and restoration ecology differ: while reintroduction focuses on a single species, restoration focuses on the ecosystem. Both are important components for the success of the American elm program. Much of this work is in progress and has not yet produced results, however, this review of current work will facilitate coordination and identify areas where further work is needed. The results of these experiments will inform managers interested in the use of American elm in restoration plantings.

Reintroduction Methods for the American Elm

Very little planting of American elm has occurred in natural systems in the last century. Thus, testing of restoration methods for DED-tolerant American elm is necessary to generate best practices for successful survival and growth and to understand any constraints on its use. Testing planting methods with bare-root and containerized stock, understanding site adaptation, and addressing other potential disease issues are important steps in identifying how to best use American elm in restoration plantings.

Operational planting methods with bare root seedlings are being tested in a study spanning multiple states. Over 4000 bare-root seedlings were planted on six riparian and floodplain forest sites to compare the performance of planted elms with other planted tree species in different site types (Haugen et al. 2017). Survival ranged from 37 to 100 percent, with herbivory by deer as a major factor limiting success. More labor-intensive methods of planting containerized stock are being tested in multiple research sites (Slavicek 2013), including floodplain forests of Ohio (Knight et al. 2012). When competing vegetation was removed at planting and controlled through mulching, large containerized trees had excellent survival and rapid growth (Slavicek 2013). The floodplain experiment showed greater survival of larger containerized trees compared to smaller containerized stock, as well as benefits of caging trees to prevent deer browsing.[2]

It is pointless to plant trees in sites where they are unlikely to survive. Experiments have been initiated to address components of site adaptation of DED-tolerant American elms, including cold tolerance, flood tolerance, and shade tolerance. Cold tolerance is being addressed in multiple experiments. Progeny from crosses between DED-tolerant American elms and Chippewa National Forest survivor trees were planted at multiple sites at the Chippewa National Forest and are being tracked for growth and survival over time (Slavicek and Knight 2012, Slavicek et al. 2009). While some progeny are growing well and demonstrating sufficient cold hardiness in this harsh environment, others regularly die back during winter months, suggesting that they lack adequate hardiness.[3] Similarly, offspring from DED-tolerant and survivor elm crosses in New England have been planted at four field trial sites in northeastern Vermont.[4] These trees will continue to be tracked for winter shoot injury and will be inoculated with DED pathogens to test for DED tolerance; the trees exhibiting both DED tolerance and ample cold hardiness will be kept in these sites to serve as a seed orchard. A second experiment in northern Wisconsin (which is testing progeny from open-pollinated DED-tolerant mother trees, a known DED-tolerant selection [Princeton], and locally-collected seeds), will also yield insights into survival of elms in a northern climate.[5] A third experiment to test cold hardiness of known DED-tolerant selections is also underway to test for differences in shoot cold tolerance among maternal lines of DED resistant stock and native paternal lines from different

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[3] Unpublished data, James M. Slavicek, Research Biologist, U.S. Forest Service Northern Research Station, 359 Main Rd., Delaware, OH 43015.

[4] Unpublished data, Christian O. Marks, Floodplain Ecologist, The Nature Conservancy Connecticut River Program, 136 West Street, Suite 5, Northampton, MA 01060.

[5] Unpublished data, Linda M. Haugen, Plant Pathologist, U.S. Forest Service, 1992 Folwell Avenue, St. Paul, MN 55108. This study is being conducted in partnership with U.S. Forest Service Northern Research Station, U.S. Forest Service Northeastern Area State and Private Forestry, U.S. Forest Service Region 9, and the Army Corps of Engineers.

plant cold hardiness zones.[6] Genetic lines with consistently greater cold tolerance could be preferentially planted in northern restoration efforts.

Other components of site adaptation, including flood tolerance, shade tolerance, and effects of competing vegetation, are also important to understand. Low seedling survival on a site with heavy flooding during the growing season (Haugen et al. 2017) raises the concern that it may be difficult to establish American elm on sites that are extremely wet. Minor variations in elevation among floodplain ecosystems can lead to very different flooding intensity experienced by planted tree seedlings. The underplanting of tree seedlings prior to harvest or mortality of canopy trees can be a successful method to establish understory trees poised to grow rapidly and fill in canopy gaps, but this strategy will only be successful if the planted seedlings are able to tolerate shading before the canopy is removed. Silvicultural guidelines list American elm as "intermediate" in shade tolerance (Myers and Buchman 1984). An experiment in floodplain forests in Ohio is examining both the flood and shade tolerance of planted American elm seedlings by testing the effects of microsite elevation and canopy openness for each of over 1000 elm seedlings planted (Knight et al. 2012). Many seedlings survived extensive spring and fall flooding; the elevation data is still being analyzed. Shade tolerance data revealed excellent survival of planted trees across a range of microsite light environments and a surprising lack of effect from competition from invasive herbaceous plants.[2]

Planting strategies for American elm may also need to consider ways to avoid risks from root grafting. While DED-tolerant elms exhibit disease tolerance when infected via beetles or stem inoculations, it is unknown how they would perform if infected via root grafts. An experiment with paired elms was initiated in 2011 to understand the risks of root grafting.[2] Inoculations will take place in several years once the trees have grown larger and formed root grafts. Additional research to understand the prevalence of root grafting in natural systems may be useful in developing planting strategies that mitigate associated risks.

It is also important to consider the "unknown unknowns"—those factors that have not yet been identified as being problematic. Two efforts have involved planting elms in many sites and tracking the trees over time. The National Elm Trials include both DED-tolerant American elm cultivars and other elm species and hybrids planted in 16 states to study growth, stress and pest resistance, and horticultural performance (Colorado State University, n.d.). Sentinel restoration sites, consisting of plantings of multiple DED-tolerant American elm selections in eight locations in four states, are being monitored to identify additional factors that may impact the success of elm restoration plantings (Slavicek 2007, 2013; Slavicek et al. 2005). These plantings may serve as an "early warning" system to identify additional pathogens of concern so that tolerance to these threats can be incorporated into the breeding program. So far, the elms are growing well at most sites. An unknown factor that was identified at one site in Ohio is a wood wasp that appears to have caused three trees to die.[3] In some Minnesota and Iowa sites, the heavy sod appears to lead to slow growth rates and high mortality of planted trees due to competition for moisture and root feeding by rodents (presumed to be the plains pocket gopher, *Geomys bursarius*).[5] Elm regeneration on the sentinel sites will be tested to understand how DED-tolerant elm may spread from plantings into surrounding landscapes.

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American Elm as a Component of Restoration Strategies

Eastern forests are impacted by many forms of disturbance, including introduced pests and pathogens, invasive plants, increases in white-tailed deer, land clearing, grazing, and climate change. American elm may be used in restoration plantings to respond to natural and anthropogenic disturbance. Its versatility and ability to thrive in a wide variety of conditions make it a useful component in both urban and natural plantings. It is one of the best species for supporting a diverse array of insect herbivores, which then support higher trophic levels including birds (Tallamy 2009). Ongoing studies are testing the use of DED-tolerant American elm in restoration plantings to address grazing in riparian areas, mineland restoration, and to compensate for ash mortality following infestation by the emerald ash borer (EAB; *Agrilus planipennis*).

Ash (*Fraxinus* spp.) filled in gaps left by dying American elm trees in many riparian and swamp areas (Barnes 1976). EAB, an invasive insect pest, now threatens ash trees in these ecosystems. In areas where ash trees are abundant, and few other trees are present in the understory or midstory, underplanting before ash mortality or replanting after ash mortality may be necessary to preserve hydric forests in some areas (Iverson et al. 2016). Multiple studies are testing progeny from DED-tolerant American elms as components of plantings in ash ecosystems to respond to the threat of EAB. A study in northern Minnesota is testing American elm and other native tree species planted in black ash wet forest ecosystems (Looney et al. 2015). Four overstory tree treatments simulated different management options: control, clearcut, group selection, and girdling to simulate mortality from EAB. A second study in riparian green ash forests in Ohio is testing underplanting of American elm and other tree species in forests affected by EAB. Early data indicate good initial survival of American elm in both studies, ranging from 32 percent to 93 percent across different overstory treatments in the Minnesota study, and 50 to 79 percent across different light levels in the Ohio study (Looney et al. 2015; Knight, unpublished data[2]).

Grazing in riparian areas can cause soil compaction, erosion, nutrient runoff, and impaired water quality (Kauffman and Krueger 1984). Limiting cattle access to sensitive riparian areas and restoring streamside habitat are strategies used to address these problems. Restoration plantings installed in 2015 at the Finger Lakes National Forest included DED-tolerant American elm selections and other native tree and shrub species that will test effects on aboveground and belowground ecosystem function.[7]

The Nature Conservancy's Connecticut River Program has planted 840 disease-tolerant American elm cultivars at a total of 33 sites in Vermont, Connecticut, Massachusetts, and New Hampshire between 2010 and 2016, with more planned for the future. These plantings include multiple DED-tolerant selections. Survival in these plantings has varied considerably from 30 to 100 percent depending on site factors such as ice flows, height and density of competing vegetation, climate, damage from voles, deer browsing, and others.[4]

American elm, along with American chestnut, is also being used in restoration plantings on reclaimed mine lands (Adams et al. 2015). Compacted and altered soils, coupled with invasive plants, present challenges in the restoration of these areas back to native forest. Additional plantings of American elm in a variety of contexts and ecosystem types suggest potential for its use in many situations. However, many plantings do not include regular data collection

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or experimental design to test specific hypotheses, and an inconsistency in data collection methods among researchers and across experiments hampers generalized data interpretation and synthesis. Follow-up data to understand the factors affecting the survival and growth of planted elm trees, as well as the success of achieving restoration goals such as ecosystem structure and function, are needed.

Discussion

Multiple research projects are underway to develop restoration methods for American elm and to develop recommendations for the use of American elm as part of a planting strategy to respond to disturbance. While little is published at the current time because many of these efforts are just beginning, within the next 5 to 10 years a wealth of information should be available. Guidelines regarding cold tolerance, flood tolerance, shade tolerance, response to competing vegetation, and root grafting will allow managers to maximize survival of planted elm trees. Experimental results from testing American elm as a component of a restoration strategy will also show how it may serve as a useful element to restore ecosystems after disturbance. One challenge in reintroduction is that successful guidelines may vary depending on specific situations and site characteristics such as forest type, competing species, local hydrology, etc. No protocol will work in all situations and preclude the need for adaptive management strategies. Managers will be able to use the DED-tolerant American elm selections and seeds, coupled with information from this research, as tools to restore forest ecosystems.

While the ongoing work will provide considerable information to guide restoration plantings of American elm, gaps in the research do exist and provide opportunities to proactively address potential challenges. As with American chestnut, both social and ecological contexts should be considered to guide American elm restoration strategies. Additional consideration of the social context will guide the formulation of goals, addressing questions such as the public perception and value of American elm in urban and forest areas, forest manager goals for incorporation of American elm, and municipal requirements for urban trees. There are also opportunities for expansion of ecological research. Because American elm has such a wide native range, plantings in additional parts of the range may be useful to identify potential problems (e.g., the sentinel sites) or test performance on different soil types and in different climates. Experiments to identify interactions among elm genetics and abiotic and biotic environmental variables may guide silvicultural, site preparation, and planting strategies. The long-term implications of American elm in restoration should be considered as well as the long-term durability of resistance to disease. Understanding the potential for the spread of DED-tolerant genes as planted trees reproduce and cross with local elms will provide researchers information needed to design landscape-scale strategies. Ultimately, a critical examination of the implications of different restoration strategies from social, policy, and ecological viewpoints will allow the program to take a more strategic approach toward the restoration of American elm.

Literature Cited

Adams, M.B.; Angel, P.; Barton, C.; Slavicek, J. 2015. **American elm in mine land reforestation.** Reclamation Matters. Fall: 34-38.

Barnes, B.V. 1976. **Succession in deciduous swamp communities of southeastern Michigan formerly dominated by American elm.** Canadian Journal of Botany. 54: 19-24.

Clark, S.L; Schlarbaum, S.E.; Pinchot, C.C.; Anagnostakis, S.L.; Saunders, M.R. [et al.]. 2014. **Reintroduction of American chestnut in the national forest system.** Journal of Forestry. 112: 502-512.

Colorado State University College of Agricultural Sciences. N.d. **National elm trial.** Ft. Collins, CO: Colorado State University. http://bspm.agsci.colostate.edu/national-elm-trial/ (accessed April 3, 2017).

Haugen, L.M.; Edge, G.; Meiers, A. 2017. **American elm reforestation trial: a partnership effort in the Upper Mississippi River watershed.** Journal of Forestry. 115(2):S102. Abstract.

Iverson, L.; Knight, K.S.; Prasad, A.; Herms, D.A.; Matthews, S. [et al.]. 2016. **Potential species replacements for black ash (*Fraxinus nigra*) at the confluence of two threats: emerald ash borer and a changing climate.** Ecosystems. 19(2): 248-270.

Jacobs, D.F. 2007. **Toward development of silvical strategies for forest restoration of American chestnut (*Castanea dentata*) using blight-resistant hybrids.** Biological Conservation. 137: 497-506.

Jacobs, D.F.; Dalgleish H.J.; Nelson, C.D. 2013. **A conceptual framework for restoration of threatened plants: the effective model of American chestnut (*Castanea dentata*) reintroduction.** New Phytologist. 197: 378-393.

Kauffman, J.B.; Krueger, W.C. 1984. **Livestock impacts on riparian ecosystems and streamside management implications: a review.** Journal of Range Management. 37: 430-438.

Knight, K.S.; Slavicek, J.M.; Kappler, R.; Pisarczyk, E.; Wiggin, B.; Menard, K. 2012. **Using Dutch elm disease-tolerant elm to restore floodplains impacted by emerald ash borer.** In: Sniezko, R.A. [et al.], tech. coords. Proceedings of the 4th international workshop on the genetics of host-parasite interactions in forestry: disease and insect resistance in forest trees. 2011 July 31–August 5; Eugene, OR. Gen. Tech. Rep. PSW-GTR-240. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 317-323.

Looney, C.E.; D'Amato, A.W.; Palik, B.J.; Slesak, R.A. 2015. **Overstory treatment and planting season affect survival of replacement tree species in emerald ash borer threatened *Fraxinus nigra* forests in Minnesota, USA.** Canadian Journal of Forest Research. 45(12): 1728-1738.

Marks, C.O. 2017. **The ecological role of *Ulmus americana* L. in floodplain forests of northeastern North America.** In: Pinchot, C.C.; Knight, K.S.; Haugen, L.M.; Flower, C.E.; Slavicek, J.M., eds. Proceedings of the American elm restoration workshop 2016; 2016 October 25-27; Lewis Center, OH. Gen. Tech. Rep. NRS-P-174. Newtown Square, PA: U.S. Department of Agriculture, Forest Service Northern Research Station: 74-98.

Marks, C.O.; Canham, C.D. 2015. **A quantitative framework for demographic trends in size-structured population: analysis of threats to floodplain forests.** Ecosphere. 6(11): article 232.

Myers, C.C.; Buchman, R.G. 1984. **Manager's handbook for elm-ash-cottonwood in the North Central States.** Gen. Tech. Rep. NC-98. St. Paul, MN: U.S. Dept. of Agriculture, Forest Service, North Central Forest Experiment Station. 11 p.

Slavicek, J.M. 2007. **Expansion of the American elm restoration effort to the upper midwest.** In: Gottschalk, K.W., ed. Proceedings, 17th USDA interagency research forum on gypsy moth and other invasive species 2006; 2006 January 10-14; Annapolis, MD. Gen. Tech. Rep. NRS-P-10. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station: 90. [Abstract].

Slavicek, J.M. 2013. **Development of methods for the restoration of the American elm in forested landscapes.** In: Sniezko, R.A. [et al.], tech. coords. Proceedings of the 4th international workshop on the genetics of host-parasite interactions in forestry: disease and insect resistance in forest trees; 2011 July 31–August 5; Eugene, OR. Gen. Tech. Rep. PSW-GTR-240. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 337-341.

Slavicek, J.M.; Baggett, K.; Swanson, G. 2009. **Restoration of the American elm on the Chippewa National Forest through generation of Dutch elm disease tolerant, cold-hardy, and site-adapted trees.** In: McManus, K.A.; Gottschalk, K.W., eds. Proceedings, 20th USDA interagency research forum on invasive species; 2009 January 13-16; Annapolis, MD. Gen. Tech. Rep. NRS-P-51. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station: 95. [Abstract].

Slavicek, J.M.; Boose, A.; Balser, D.; Cavender, N. 2005. **Restoration of the American elm in forested landscapes.** In: Gottschalk, K.W., ed. Proceedings, 16th USDA interagency research forum on gypsy moth and other invasive species; 2005 January 18-21; Annapolis, MD. GTR-NE-337. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northeastern Research Station: 74. [Abstract].

Slavicek, J.M.; Knight, K.S. 2012. **Generation of American elm trees with tolerance to Dutch elm disease through controlled crosses and selection.** In: Sniezko RA [et al.], tech. coords. Proceedings of the 4th international workshop on the genetics of host-parasite interactions in forestry: disease and insect resistance in forest trees; 2011 July 31-August 5; Eugene, OR. Gen. Tech. Rep. PSW-GTR-240. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 342-346.

Steiner, K.C.; Westbrook, J.W.; Hebard, F.V.; Georgi, L.L.; Powell, W.A.; Fitzsimmons, S.F. 2016. **Rescue of American chestnut with extraspecific genes following its destruction by a naturalized pathogen.** New Forests. 48(2): 317-336.

Tallamy, D.W.; Schropshire, K.J. 2009. **Ranking lepidopteran use of native versus introduced plants.** Conservation Biology. 23(4): 941–947.

Wheeler, N.C.; Steiner, K.C.; Schlarbaum, S.E.; Neale D.B. 2015. **The evolution of forest genetics and tree improvement research in the United States.** Journal of Forestry. 113(5): 500-510.

RESTORING ECOSYSTEM RESILIENCE TO URBAN FORESTS USING DUTCH ELM DISEASE-TOLERANT AMERICAN ELM TREES

Charles E. Flower, Cornelia C. Pinchot, and James M. Slavicek[1]

Extended Abstract.—Urban forests contribute significantly to human health and environmental quality (Sanesi et al. 2011). As such, maintaining healthy urban forests resilient to pollution (atmospheric and soil), high temperatures, compacted soils, and poor drainage is critical. However, these forests have been hard hit by development, pests, and pathogens, consequently reshaping their diversity, structure, and resilience.

Pests and pathogens either selectively target specific genera (e.g., Dutch elm disease and emerald ash borer) or indiscriminately kill a variety of host species (e.g., Asian longhorned beetle). Many of the trees commonly targeted by pests and pathogens are (e.g., maple) or historically (e.g., elm and ash) were widely distributed across urban areas. Such disturbances reduce native tree diversity and compromise the ecosystem services delivered by forests (both urban and rural), negatively impacting their resilience to future outbreaks and climate change (Flower and Gonzalez-Meler 2015). This could have considerable economic implications for municipalities (Kovacs et al. 2010).

Reintroducing newly cultivated DED-tolerant selections of American elm across the urban-rural gradient in tandem with suitable understory species will enhance the long-term resilience of these systems by increasing the genetic diversity of elm and enhancing the functional redundancy in these systems.

Research efforts are underway to bring the once-dominant American elm back into the urban landscape where it was once ubiquitous. Urban foresters and citizens are again planting American elm trees, yet widespread availability of American elm is limited to a handful of cultivars: "Princeton", "Accolade", and "Valley Forge" (Giblin and Johnson 2017). Enhancing the genetic diversity of American elm genotypes used in urban forests is essential to maintaining durability of Dutch elm disease (DED) tolerance and the resilience of urban forests. U.S. Forest Service researchers have cultivated more than 100 American elm selections, many of which exhibit DED tolerance and may be suitable for urban and rural restoration plantings (Flower et al. 2017). Research has been initiated in Columbus, OH to simultaneously test the suitability of these selections in urban plantings, methods for reintroducing elm across the rural-urban gradient, and interactions among restoration treatments and ecosystem dynamics.

To complement the existing forest restoration plantings around the Midwest and northeastern United States (Knight et al. 2017), we are initiating a project to simultaneously test methods for reintroducing elm across the rural-urban gradient as well as the interactions among restoration treatments and ecosystem dynamics using a network of sites in the Columbus, Ohio, metropolitan area. The diversity in habitat types ranging from degraded riparian forests to urban street plantings in and around Columbus will allow us to test restoration approaches across a variety of habitats. These treatments will span a gradient of land-use intensity and associated levels of pollutants, impervious surface, community uses, etc. Restoration treatments on degraded riparian forest and abandoned agricultural lands will include 1) invasive species

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removal; 2) invasive species removal in combination with multi-species tree and shrub plantings; and 3) control. We will use multiple elm selections to address the lack of elm diversity across the urban forest. Results from this study will offer guidance for introducing DED-tolerant elm selections into urban forests, provide restoration strategies for degraded areas, and enhance our understanding of the ecological functions provided by American elm.

LITERATURE CITED

Flower, C.E.; Gonzalez-Meler, M.A. 2015. **Responses of temperate forest productivity to insect and pathogen disturbances.** Annual Review of Plant Biology. 66: 547-569.

Flower, C.E.; Slavicek, J.M.; Lesser, D.; Eshita, S.; Pinchot, C.C. 2017. **Canopy decline assessments in American elm after inoculation with different doses of *Ophiostoma ulmi* and *O. novo-ulmi*.** In: Pinchot, C.C.; Knight, K.S.; Haugen, L.M.; Flower, C.E.; Slavicek, J.M., eds. Proceedings of the American elm restoration workshop 2016; 2016 October 25-27; Lewis Center, OH. Gen. Tech. Rep. NRS-P-174. Newtown Square, PA: U.S. Department of Agriculture, Forest Service Northern Research Station: 24-29.

Giblin, C.P.; Johnson, G. 2017. **Pruning cycles and storm damage: are young American elms failing prematurely?** In: Pinchot, C.C.; Knight, K.S.; Haugen, L.M.; Flower, C.E.; Slavicek, J.M., eds. Proceedings of the American elm restoration workshop 2016; 2016 October 25-27; Lewis Center, OH. Gen. Tech. Rep. NRS-P-174. Newtown Square, PA: U.S. Department of Agriculture, Forest Service Northern Research Station: 122-132.

Knight, K.S.; Haugen, L.; Pinchot, C.; Schaberg, P.; Slavicek, J.M. 2017. **American elm in restoration plantings: a review.** In: Pinchot, C.C.; Knight, K.S.; Haugen, L.M.; Flower, C.E.; Slavicek, J.M., eds. Proceedings of the American elm restoration workshop 2016; 2016 October 25-27; Lewis Center, OH. Gen. Tech. Rep. NRS-P-174. Newtown Square, PA: U.S. Department of Agriculture, Forest Service Northern Research Station: 133-140.

Kovacs, K.F.; Haight, R.G.; McCullough, D.G.; Mercader, R.J.; Siegert, N.W.; Liebhold, A.M. 2010. **Cost of potential emerald ash borer damage in U.S. communities, 2009-2019.** Ecological Economics. 69: 569-578.

Sanesi, G.; Gallis, C.; Kasperidus, H.D. 2011. **Urban forests and their ecosystem services in relation to human health.** In: Nilsson, K. [et al.], eds. Forests, trees and human health. Dordrecht, The Netherlands: Springer: 23-40.

2016 WORKSHOP ATTENDEES

Photo by Kathleen Knight, U.S. Forest Service.

Front row, left to right: Kirsten J. Lehtoma, Danielle K. Martin, Stephanie M. Adams, Kris R. Bachtell, Keith E. Woeste, R. Jay Stipes, Steven (Steve) M. Eshita, James (Jim) M. Slavicek, Linda M. Haugen, Cornelia (Leila) C. Pinchot, Garrett L. Beier, and Timothy (Tim) D. Fox.

Middle row, left to right: Kathleen S. Knight, Michael Marcotrigiano, Kimberly (Kim) Shearer Lattier, Johanne Brunet, Sandra (Sandy) L. Anagnostakis, Cristina Rosa, Rakesh Minocha, Stephanie Long, Susan E. Bentz, Makund R. Shukla, Raymond (Ray) P. Duval.

Back row, left to right: Louis Bernier, Christian O. Marks, Aziz Ebrahimi, Alan T. Whittemore, William (Bill) L. MacDonald, Raymond (Ray) P. Guries, Chad P. Giblin, Bruce R. Fraedrich, Ryan L. Murphy, Nancy L. Hayes-Plazolles, Tom Zetterstrom, Charles (Charlie) E. Flower, David (Dave) W. Carey, Sherif M. Sherif.

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WORKSHOP AGENDA

American Elm Restoration Workshop October 25th - 27th, 2016	
Tuesday, October 25th	
James (Jim) M. Slavicek, Kathleen S. Knight, Cornelia (Leila) C. Pinchot, Charles (Charlie) E. Flower, Nancy L. Hayes-Plazolles, Kirsten J. Lehtoma, Rakesh Minocha, Stephanie Long, Keith E. Woeste, Paul G. Schaberg, Shiv Hiremath, USDA Forest Service, Northern Research Station (NRS); Christian O. Marks, The Nature Conservancy	Welcome, USDA Forest Service and The Nature Conservancy Collaborative Research Project on Restoration of American Elm
Michael Marcotrigiano, Smith College	An overview of historic work on DED and American elm
Dutch Elm Disease (DED) Pathogens	
Louis Bernier, Centre for Forest Research, Laval University	A genome-wide approach to the study of parasitic fitness and evolution in the Dutch elm disease fungi
DED Tolerance: What is it? How do you quantify it? What are the genetics?	
James (Jim) M. Slavicek, USDA Forest Service, NRS	DED tolerance of progeny trees from crosses of DED tolerant selections and survivor elms
Louis Bernier, Centre for Forest Research, Laval University	Identifying *Ulmus americana* genes that are induced in response to Dutch elm disease
Sherif M. Sherif, Virginia Agricultural Research and Extension Centers, Virginia Polytechnic Institute and State University	Analysis of American elm transcripts and transcriptomes reveals novel insights into the location and mechanism of tolerance to Dutch elm disease
Alan T. Whittemore, USDA Agricultural Research Service (ARS), US National Arboretum	Ploidy and cryptic genetic diversity in *Ulmus Americana*
Garrett L. Beier, Department of Plant Pathology, University of Minnesota	The effects of inoculation with *Ophiostoma novo-ulmi* on water conductance in *Ulmus americana* with varying levels of resistance to Dutch elm disease
Garrett L. Beier, Department of Plant Pathology, University of Minnesota; Raymond (Ray) P. Guries, Department of Forestry, University of Wisconsin; James M. (Jim) Slavicek, USDA Forest Service, NRS; and Susan E. Bentz, ARS, National Arboretum	Panel: Challenge inoculation. Discuss methods used by various researchers, how to standardize results
Tom Zetterstrom, Elm Watch; Bruce R. Fraedrich, Bartlett Tree Experts; and Chad P. Giblin, Department of Forest Resources, University of Minnesota	Lessons from the street: Considerations when selecting/breeding elms for the urban environment
All	Group discussion: development of an elm cultivar table

American Elm Restoration Workshop October 25th - 27th, 2016	
Wednesday, October 26th	
About elm	
Jennifer L. Koch, USDA Forest Service, NRS	Long term trends in elm density in the eastern United States related to DED and elm yellows
Johanne Brunet, USDA ARS and The University of Wisconsin	Elm genetic diversity and hybridization in the presence of Dutch elm disease
Alan T. Whittemore, USDA ARS, US National Arboretum	Molecular phylogeny of the genus *Ulmus*, and how it relates to potential sources of resistance to DED
Elm yellows	
Gary W. Moorman, Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University	Elm yellows at Penn State
Cristina Rosa, Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University	Research developments on the elm yellow epidemic at Penn State
	Group discussion: Elm yellows
Other Ways to combat DED	
William (Bill) L. MacDonald and Mark L. Double, Division of Plant and Soil Sciences, West Virginia University	The Glenwood Estate-Our thirty-two year experience using Arbotect® to control DED
Topical Discussion	Acquired resistance—Dutch Trig and related concepts—do they work for American elm?
Tour of USDA Delaware Forestry Sciences Laboratory and plantation	
James (Jim) M. Slavicek, USDA Forest Service, NRS and NRS Staff	Tour the greenhouse and field plantings
Chad P. Giblin, Department of Forest Resources, University of Minnesota; Tom Zetterstrom, Elm Watch; and Bruce R. Fraedrich, Bartlett Tree Experts	Field discussion/demo on form of elm cultivars - recognition, correction by pruning
Thursday, October 27th	
American elm in forests and urban areas: restoration of an iconic tree species	
Christian O. Marks, The Nature Conservancy	The ecological role of American elm in floodplain forests of northeastern North America
Kathleen S. Knight, Cornelia (Leila) C. Pinchot, James (Jim) M. Slavicek, USDA Forest Service, NRS; and Linda M. Haugen, USDA Forest Service, Forest Health Protection	A strategic approach to restoration of American elm in natural areas
Topical Discussion	Cultivars: Discussion of the table/spreadsheet of cultivar information that we have been building over the last two days
Future direction in American elm Breeding	
Topical Discussion	Elm breeding and genetics… what are our best "next steps"?
Topical Discussion	Discussion of bin items

Pinchot, Cornelia C.; Knight, Kathleen S.; Haugen, Linda M.; Flower, Charles E.; Slavicek, James M., eds. 2017. **Proceedings of the American elm restoration workshop 2016**; 2016 October 25-27; Lewis Center, OH. Gen. Tech. Rep. NRS-P-174. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station. 148 p.

Proceedings from the 2016 American Elm Restoration Workshop in Lewis Center, OH. The published proceedings include 16 papers pertaining to elm pathogens, American elm ecology, and American elm reintroduction.

KEY WORDS: *Ulmus, Ophiostoma*, Dutch elm disease, elm yellows, phytoplasma, tree species restoration, tree breeding, disease resistance, forest pathogens